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More trouble for the hapless shuttle

Ironically, the shuttle device for putting objects into orbits about the Earth is being threatened now by commercial as well as technical problems (see page 789). After a decade of planning, much of it imaginative and some of it over-imaginative, it now appears that the shuttle may not be able to hold its own commercially with other means of launching satellites. The European rocket Ariane appears, in the short run at least, to be able to offer launching facilities more cheaply than comparable rocket systems in the United States, and there is at least a possibility that the shuttle itself will not be much cheaper (see page 789). In any case, the shuttle is so far behind schedule, and demands from the Pentagon on its services so clamant, that it may be some time before the device is able to perform the general service for which it was designed — making sure that the world is well supplied with telecommunications satellites (on which it is now dependent), assisting with the exploration of the Solar System and the regions beyond and giving all of us the sense that space, as we have learned to call it, is part of our domain. The irony is, of course, that the shuttle, intended as a way of cheapening the launching of satellites, should appear to be threatened by an upstart competitor from Europe. Especially in France, people will be recalling the legend of David and Goliath. The snag is that, in the end, we shall all be losers.

A device such as the shuttle (intended to have its second trial flight next month) is not merely desirable but essential. When the National Aeronautics and Space Administration decided, at the end of the Apollo programme, that sending a man to Mars would be a less useful application of the skills that it had acquired than the building of a reusable launching system, it took the right decision. Better to go step by step than to hunt for yet another worldwide television hook-up. But the shuttle, as it has been developed, is something of a bastard. The original conception of a launching device that could be used over and over again has been adulterated by arranging that the largest items of equipment, the launching rockets, should fall into the sea and be lost. Some time in the coming decade, after the system as it stands has proved itself, attention may be turned again to the recovery of these items. In the meantime, there is a serious danger that the shuttle will limp along, working principally for the military, and that the usefulness of Earth satellites will be denied.

In all the circumstances, it is necessary that the space administration, or perhaps even the US Congress, should stand back from the problems that now afflict the shuttle and ask where, in the end, it is meant to go. The issues that must be settled are not simple, but neither are they beyond comprehension.

First, there is a case for asking whether the present concept of the shuttle, as embodied in the spacecraft called "Columbia", is the most appropriate, given what has been learned in the past ten years; if it is not, the space administration (or Congress) should be prepared to write off the huge expenditures of the past decade, and to start again. Such a question could be settled only after a careful technical enquiry, but this is the time at which to make it. Second, there should be a more sober appraisal than there has been so far of the utility of being able to put satellites into orbits — a futuristic appraisal of the market. For the past several years, telecommunications authorities have been assuming that there will always be some way of launching their relay stations, and that the cost will be a small part of the cost of providing them with signals; in present circumstances, in which the benefits of terrestrial switching circuits are only just becoming clear, is that assumption still valid? And, since it would be not merely a black mark for the shuttle project but an international tragedy that the Large Space Telescope should not be launched, or not launched more or less on time, how should such projects be organized? Finally, there are questions about the future, and of several kinds. Do the difficulties that have afflicted the shuttle in

the past few years imply that it has been discovered empirically — the hard way — that even the United States is no longer capable of everything? What in the medium term, say by the end of the century, is the utility of the Earth orbit? Science and the Pentagon apart, telecommunications will have to bear the burden. And what of the more distant future? Science fiction, which has a habit of becoming fact, would suggest that the time will come when people will think it intolerable that they should be prevented by the lack of facilities from going where they choose. Such conceits, always infuriating for those concerned with next year's budget, are nevertheless historically (so far) undeniable.

Agony in London

Whom the gods would destroy, they first make seem ineffectual. This seems to be the underlying trouble at the University of London, which has just been through the traumatic process of dividing the public grant for the present academic year (which began financially on 1 August) among its several schools and specialized institutions. But the university, which is a microcosm of the British university system as a whole, is not entirely to blame. Both the university and the University Grants Committee earlier in the year were faced with an impossible task — that of saving large amounts of money so quickly that they could not properly take account of academic and educational considerations. Solomon himself would have been hard pressed to make an equitable division of the diminished budget. Yet both the university and the grants committee have responded with delay. The grants committee let its dependants know only on the eve of the academic year what they would have to spend in the three succeeding years. The University of London, in which financial decisions are delegated to a body called the Court, has delayed a further six weeks and has announced financial allocations (*Nature*, 20 August) which are inconsistent both with the principles laid down by the grants committee and with the interim recommendations of the committee (under Sir Peter Swinnerton-Dyer) which the university had set up to suggest how its organization might be adapted to much smaller budgets.

What has happened, and is about to happen, at the University of London is important because it is among the most important of the universities in the United Kingdom. It has more students than any other British university, and is comparable in size with the Berkeley campus of the University of California. The university is the largest single source of physicians in the United Kingdom, but also has specialized institutions for training architects, veterinarians, pharmacists and even agriculturalists. Constitutionally, however, the university is not an integrated whole but an agglomeration of titularly autonomous institutions so loosely held together that they are a federation on the Canadian pattern, not that of the United States. The separate institutions include a dozen medical schools (soon to be fewer), internationally known centres of teaching and scholarship such as the London School of Economics and Imperial College, a host of graduate institutions (not only medical) whose importance nationally and internationally is beyond dispute and no fewer than eight more general colleges, scattered over twenty-five miles, which differ in size, the pattern of courses offered to undergraduates and their reputations in research. The question now is whether last week's decision by the Court will have



irreparably damaged the character of this remarkable, if anomalous, institution.

On at least three counts, the Court's decisions cannot fail to cast a long shadow over the university. First, the Court seems meekly to have accepted the grants committee's edict that the proportion of students (graduates and undergraduates) following science courses should be reduced by 11.3 per cent by the academic year beginning in 1983. This is a sharper contraction than the intended reduction of the British university student population as a whole and much sharper than the planned contraction of science teaching — a mere 2.2 per cent throughout the United Kingdom. Taking the wording of the grants committee's letter literally, however, the Court of the university has decided that teaching in the physical sciences should be maintained, and has gone on to plan for a reduction of 28 per cent in the numbers of students following courses in biology. The grants committee's recommendation that special consideration should be given to biology courses of "potential economic importance" (another name for biotechnology?) seems not everywhere to have been heeded. The result, by 1983, is likely to be that most departments of "soft" biology in the university will have shrunk to such a size that they are no longer viable. In the meantime, most of them will no doubt whistle hard to keep their spirits up and will admit more students in the next few days than prudence would dictate.

The second change of pattern now decreed that will prejudice the educational work of the university as a whole is the Court's decree that undergraduate courses in which two principal subjects are combined should bear the brunt of the contraction. To be fair, the Court has not dreamed up this conclusion of its own accord, but instead has asked the grants committee for "guidance". Having been told of what was plainly an afterthought by the committee, the court has done its arithmetic accordingly. At no point has it been considered whether these decisions are educationally desirable. Yet there are several schools within the university where combined courses of this kind, involving both general and particular studies, are much valued by employers, not to mention students. In any case, when it is plain that one of the faults of the British university system is that it offers too specialized an education to most undergraduates, by what right does the grants committee recommend that the efforts made in London to put that balance right should be negated? The Court says in its letter to its pensioners that "the far-reaching implications of this [decision] will have to be examined within the university". The snag is that its financial decisions for the coming year will have preempted sensible discussion.

Third, the allocations of funds from the university budget of £157 million for the coming year must put the survival of many of the smaller schools of the university in jeopardy. The difficulty is that institutions whose costs consist largely of salaries for academic and ancillary staffs — often three-quarters of the total — cannot hope to live within the shorter commons now decreed by getting rid of staff, for contracts of employment often require that notice of dismissal should be given before the beginning of an academic year. So how will such places manage? Especially in those schools where the numbers of students from overseas has fallen sharply, the outlook must be grim. Yet the Court of the university promises nothing in the way of help. Instead it hopes that the "cost of contraction" can be met "from some special source" and mentions the £20 million the University Grants Committee set aside last year to meet the cost of academic redundancy. On present form, the true cost will be far greater.

For other than financial reasons, this is an awkward time for the university. The Vice-Chancellor, Lord Annan, is about to be succeeded (on 1 September) by Professor Randolph Quirk. The senate of the university, responsible for academic policy, has just been reconstituted and will not be meeting until November. The new management has a daunting task ahead of it. It will also have an awkward constitutional problem to tackle. The Court of the university is charged with making final decisions of the distribution of funds but is barred from making decisions with academic implications without consulting the academic senate. The past few weeks have shown is that, at a time of rapid

contraction, the notion that academic and financial considerations can be cleanly separated is a fiction. But so it must be even in good times. The isolation of the Court's proceedings from the rest of the university, which has made a nonsense of the committee on academic organization, should not continue.

The university must recognize that the looseness of its constitution is increasingly an encumbrance. In spite of the committees which occupy a substantial part of many academics' time, the educational policy of the university is an amalgam of the separate decisions of individual schools. That it could or should be otherwise at places such as the London School of Economics or at Imperial College is unthinkable, but in the university as a whole, the result is inevitably duplication and incoherence. If it had the time, a stronger university might be able to create a pattern that makes more sense. But even in the short run, a stronger university could help deal with the most urgent problem — the future of academic staff. For it would be thoroughly anomalous if some parts of the university found themselves firing, or retiring prematurely, academics who might be usefully employed elsewhere within the university. And it is inequitable that the people who will be put out of work should be those who happen to be employed at schools which have been hard hit, either by the disappearance of overseas students or by the decisions forced on the Court by the arithmetic of penury — and the last-minute guidance from the grants committee.

Year of the Medfly

Californians (many of whom are ethnically Chinese) may remember 1981 as the "year of the Medfly". With a little luck, they may also come to think of 1981 as a year in which the innocence of those who pretend that nature is never malevolent was made to fade away.

The Medfly is an aphid, and its name is arrived at by a shortening of "Mediterranean". It will probably never be possible to tell how the fly reached California or the importance, in the infestation of the past few weeks, of the supposed infertility of the supposedly sterile males released by the state government into the wild. With the winter (such as it is) soon to come, Californians will with reason be hoping that most, perhaps all, of this exotic species will be killed off, and that that will be an end of the affair. Most fruit farmers will be worse off, some may even have been made bankrupt, but life will go on, on Sunset Boulevard and elsewhere.

Unfortunately, the truth is different. Aphids are pests and the stringent quarantine regulations in the United States that prohibit the unchecked importation of citrus fruit make the United States especially vulnerable to unfamiliar pests such as the Medfly. One concomitant of policies based on quarantine is that the consequences of a breakdown must be draconian. In Britain, for example, where vaccination against foot-and-mouth disease is not practised, infected animals must be slaughtered; and potential harbingers of rabies must be kept in isolation for six months. In California, the recognition of the first Medfly should have been followed by a vigorous attempt at its eradication.

Governor Jerry Brown was, by all yardsticks, slow to come to this conclusion. Everybody will sympathize, although not everybody will condone. In many ways, it is admirable that California should have taken the lead, over many years, in the protection of its own delectable environment. Governor Brown himself, one of the torch-bearers, has benefited politically as a result. Much of what has been done in California to reduce the concentration of nitric and nitrous oxides in the atmosphere has cost a great deal of money, but somehow the state (and Mr Brown) has scraped along. The trouble with the Medfly is that it has challenged the governor in a way that could not fail to be politically embarrassing. Nobody who wants to stay in Sacramento can, with an easy conscience, order out the spray guns. So the Medfly has prospered. It would be sad if Governor Brown's fortunes, as a result, went into decline.

Biotechnology's first casualty — so far

DNA Science left out on a limb

Washington

E.F. Hutton, one of Wall Street's leading investment houses, is having second thoughts about its involvement in recombinant DNA research. Earlier this month the company backed out of a deal it had set up which would have used money from over 40 institutional investors to support academic research in both US and foreign laboratories. Now it is talking of an alternative, less ambitious scheme exploiting recent changes in tax legislation to sell genetic engineering research as an attractive tax shelter.

The initial plan, first announced in February of this year, was to channel investment funds to scientists through a company set up by Hutton called DNA Science. The centrepiece of this scheme was an agreement with the Weizmann Institute of Science in Israel for collaboration on research projects. Weizmann would have provided the molecular biologists and DNA Science the chemistry through Nobel laureate Dr Christian B. Anfinsen, appointed chief scientist of an Israeli subsidiary called Taglit.

According to Hutton, the deal fell apart when the investment company began to have doubts about the legal propriety of mixing "passive" and "active" investors, some merely seeking a financial return, others demanding a greater involvement. The potential conflict between these goals surfaced when one proposed investor, Johnson and Johnson, began negotiating for exclusive marketing rights to any products which resulted from DNA Science-financed research.

Failure to reach agreement before a target date stipulated in the publicly-distributed prospectus, by which \$40 million was to have been raised, meant that Hutton was required to return the money it had already collected from outside investors. But the debate over the appropriate form of involvement by investors also reflects growing concerns that the potential commercial benefits of genetic engineering may have been oversold.

In addition to dropping the Weizmann deal, which would have focused largely on interferon, other proposals now being reassessed include an arrangement with the Battelle Memorial Institute in Columbus, Ohio, for research into projects such as the use of microorganisms to degrade toxic wastes, and collaboration with Dr John Baxter of the University of California

in San Francisco.

Dr Harsanyi, vice-president of DNA Science, says the company will now concentrate on putting together individual projects or "packages of projects" for separate funding by groups of investors, rather than trying to raise the initial capital in one lump. This means shifting the focus from long-range research to projects with more clearly-defined commercial goals.

Exploiting the tax haven incentives offered by research and development financing that were recently increased by the federal government will exclude some of the original investors, such as pension funds, which already enjoy significant tax concessions.

Ironically Hutton was one of the first major financial houses to promote the long-range commercial potential of recombinant DNA research. Nelson M. Schneider, a pharmaceutical analyst with the company who is credited as the principal architect of DNA Science and is now one of its vice-presidents, reported to a congressional committee in June that, if present trends continue, by 1985 private companies could be putting as much into biomedical research as are the National Institutes of Health (NIH).

Under the scheme unveiled in February, which had been suggested as a model for university/industry collaboration in supporting recombinant DNA research,

DNA Science would have set up a number of small "daughter companies" near university campuses in which university staff could apply themselves to commercially-oriented research while maintaining their campus links.

This was to have been the arrangement with the Weizmann Institute under an agreement reached with the institute's commercial arm, the Yeda Research and Development Corporation. Dr Anfinsen said last week that he had resigned from his post as head of the laboratory of chemical biology at the NIH's National Institute of Arthritis, Metabolism and Digestive Diseases to take up the Israeli position with DNA Science. He said that he was waiting to see what happened at the Weizmann Institute, which might continue working in the same direction on its own, before deciding what to do next.

DNA Science officials are confident that much of their work in finding both investors and research projects needing support can be salvaged. Dr Harsanyi says that several university groups have approached Hutton, interested in exploiting their research, but reluctant to become involved in the commercial management of their results. DNA Science is offering to take on this management responsibility, which could include finding potential customers for products.

David Dickson

CERN declares peace with the locals

The 26-km circumference large electron-positron ring (LEP) proposed for construction at CERN near Geneva seems to be steering itself successfully among the several political shoals that surround the project. The latest development is that CERN and the most active environmental opposition group, Agena, have issued a mollifying joint statement.

Agena, which includes a local mayor among its six members, was successful in halting work on a reconnaissance gallery by means of a legal technicality. It has also been active in canvassing support against LEP within the Jura region of France, so any degree of rapprochement between Agena and CERN is significant.

In the statement, Agena says it is concerned about LEP polluting water and the air, and about LEP's possible effect of reducing employment at CERN (to help pay for the project). Agena asks that CERN should adopt a "different approach" and give local people time to express their opinions on the project.

For its part, CERN promises to consult all relevant associations and trade unions in "an atmosphere of cooperation"; says that it "understands" Agena's fears; that anyone is free to speak to CERN and its director-general, Professor Herwig Schopper, and that no work will be done

until an environmental impact statement is complete, and the French and Swiss authorities have been given time to react through the normal national channels providing planning permission.

This latter move is an important step for CERN — although Schopper proposed it at the June meeting of CERN Council, well before the joint statement with Agena. Legally, CERN is an international organization which officially needs to deal only with the foreign ministries of member and host states. But construction of LEP, reaching deep into French and Swiss territory far from the original CERN site, has occasioned new rules.

The principal step is the production of an environmental impact statement for the French Ministry of Research and Technology, the Ministry of Environment and the Ministry of External Relations in October. Then, under French law, a commission of inquiry will be set up which will assess local opinion and invite witnesses (including, presumably, Agena itself). The commission will report, and finally a decision will be taken either by the local *préfet* of the *département* or the *Conseil d'Etat*. This process could take until March 1982. Swiss law is less exacting, but the French process is likely to be longer.

Meanwhile, the French research ministry

is taking steps to unblock the restraint on the LEP reconnaissance gallery, which depends on whether the gallery is a temporary or a permanent construction. If it is temporary, the law under which permission was originally granted is appropriate; if permanent — as Agena successfully argued — it is not. The ministry is attempting on the one hand to argue that the gallery is, after all, temporary and on the other to get approval through a different law. **Robert Walgate**

Particle physics

Director resigns

Washington

Dr George Vineyard, director of the Brookhaven National Laboratory on Long Island, New York State, announced last week that he is stepping down in order to return to full-time research. His resignation has been accepted "with regret" by the board of trustees of Associated Universities Inc., the consortium of nine universities which operates the Brookhaven Laboratory for the US Department of Energy. In a statement issued after the board's meeting last Monday, Dr Vineyard said that after nine years as director of Brookhaven, where he has been since 1954, "this appeared to be a good time to make the move".

His resignation has, however, inevitably been linked to growing speculation that the Reagan Administration is contemplating cutting funds for further development work on Brookhaven's planned 400×400 GeV intersecting storage accelerator (ISABELLE) which is facing delays and cost over-runs.

Uncertainty over the fate of ISABELLE has been growing ever since the laboratory encountered serious difficulties in developing the superconducting magnets, 1,100 of which will be required to complete the accelerator's two-mile circumference ring (*Nature* **286**, 435; 1980).

Brookhaven now says that the problems with the magnets seem to have been solved with a new design, but the delays have inevitably resulted in cost over-runs. The bill for ISABELLE is now put at \$500 million, compared with an original estimate of \$420 million, and completion is now expected in 1988 rather than 1986.

Given the intense competition from the new proton-antiproton collider under construction at CERN in Geneva, which is now likely to beat ISABELLE to one of its principal goals, the discovery of weak vector bosons, those responsible for ISABELLE face a daunting task in maintaining political support for their machine.

Dr Vineyard denies that his resignation has anything to do with the problems with ISABELLE, pointing out that the accelerator has "turned the corner by overcoming technical difficulties with the

superconducting magnets". He says that other research at the laboratory is in a healthy state, and that the new national synchrotron light source is about to go into operation.

The Reagan Administration has, however, been making lukewarm noises about continued support. A meeting between the High Energy Physics Advisory Panel and the National Science Foundation next month is likely to discuss options. One is a scaled-down design, using superconducting magnets which have already been constructed at the Fermi National Laboratory but produce a slightly lower field that would mean a lower luminosity. Another is to scrap ISABELLE altogether. The Administration is awaiting the outcome of this meeting before deciding whether to go on as planned, or adopt one of these alternatives.

David Dickson

Incoherent scatter radar

Auroral visions

A new high-power radar facility, designed to study the Earth's upper atmosphere and magnetosphere at auroral latitudes, was inaugurated by the King of Sweden on 26 August. The £13 million radar's operation will be supervised by the European Incoherent Scatter Association (EISCAT) whose headquarters are in Kiruna, Sweden. The director of the association is Tor Hagfors.

The member countries of EISCAT are West Germany, France, the United Kingdom (each contributing 25 per cent to the capital and running costs), Sweden, Norway (both 10 per cent) and Finland (5 per cent). Half the working time of the facility will be spent collecting data for common use. For the remainder, each country will have control of the facility for a time proportional to its financial contribution.

The radar was originally scheduled to start operation in 1978 but was delayed due to a faulty klystron — a vital transmitter component which turned out to be more difficult to develop than expected. The delay must have proved particularly frustrating to those itching to make their reputations with the new facility, but EISCAT's twenty or so staff have spent the time making astronomical observations with the receivers and developing computer software.

The facility consists of two radar systems, one operating at VHF (224 MHz) which is expected to be ready by next year, and a tristatic UHF (933 MHz) system with a transmitter/receiver at Tromsø, Norway, and receivers at Kiruna and Sodankylä, Finland. The UHF system has already produced its first crop of data. The combined systems will be able to monitor the atmosphere from 50 to 3,000 km.

The incoherent scatter technique, which EISCAT's radar utilizes, exploits the fact

Deadline missed

Legislation to bring Britain into line with the European Commission's directive on the notification of new chemicals will not now be enacted before the 18 September deadline. The Health and Safety Executive acknowledged this week that too many problems still remain to be ironed out. And in any case, Parliament will not reconvene until October.

The executive is not, however, worried that the commission will take proceedings against the United Kingdom — the United Kingdom is not the only laggard. One source of confusion is that the European directive requires the compliance not merely of governments but of other corporate bodies, including commercial companies, which may find that after 18 September they are bound by the directive.

The commission is planning to publish in the near future an inventory of all chemicals at present manufactured and sold in Europe. Companies will have nine months from the date of publication to tell the commission of substances not on the list. After that, new substances will have to be tested (toxicologically and otherwise) in accordance with the directive. Until then, however, the British and other European parliaments will have a breathing space in which to find time to comply.

Judy Redfearn

that radio waves can be scattered off individual electrons and off density variations caused by plasma waves in the ionosphere. This region of the atmosphere, which is ionized by solar and cosmic radiation, extends upwards from 50 km or so. The spectrum of backscattered radio pulses can be analysed to reveal a surprisingly large number of atmospheric parameters such as electron and ion densities, ion composition, electric fields, neutral and ionized wind velocities and temperatures.

Several incoherent scatter facilities have been constructed in the past, the most powerful being at Arecibo in Puerto Rico. Only one has operated at auroral latitudes, that at Chatanika in Alaska, which is itself to be moved to Greenland to allow more effective collaboration with EISCAT. The auroral zone is particularly important to those interested in solar-terrestrial relations because it is here that those relations are most manifest. For example, charged particles ejected from the Sun during solar disturbances can be trapped in the Earth's magnetosphere and may eventually spiral down the Earth's magnetic field lines to precipitate into the auroral upper atmosphere, causing heating and intense electric currents. EISCAT's sensitive radar should allow a detailed picture of such events to be developed, particularly when used in conjunction with other ground, rocket and satellite based experiments.

Philip Campbell

Soviet academics

Three deprived

Three Soviet Jewish refusenik scientists have recently been deprived of their higher degrees, apparently as a result of their wish to emigrate to Israel. They are Aleksandr Paritskii, a Candidate of Technical Sciences, formerly employed at the Khar'kov Institute of Metrology on the design of high-precision ultrasonic ranging devices; Valerii Soifer, from Moscow, a Candidate of Biology, specializing in plant mutagenesis; and Mikhail Fuks-Rabinovich of Moscow, a meteorologist.

Paritskii and Soifer, who applied to go to Israel in 1976 and 1977 respectively, were refused permission on the grounds of the "secrecy" of their former employment. The reason that Fuks-Rabinovich did not receive his visa is unknown.

Higher degrees — those of "Candidate" (roughly equivalent to a PhD) and "Doctor" (which normally requires a minimum of ten years further research) — are not, in the Soviet Union, conferred by individual universities, but are instead awarded by a special all-union body, the Higher Attestation Commission (*Vyssшая Attestatsionnaya Komissiya*; VAK).

Originally VAK came under the Ministry of Higher and Specialized Secondary Education, but was transferred in 1974 to the control of the Council of Ministers by a resolution of the Central Committee of the Communist Party and the Council of Ministers of the Soviet Union. This involved what the chairman of VAK, Professor V.G. Kirillov-Ugryumov, described as "qualitative changes". In particular, he said, the new regulations required postulants for a higher degree to "combine a profound professional knowledge with a mastery of Marxist-Leninist theory and with the convictions of an active builder of communist society".

Jews who wish to emigrate to Israel clearly do not show such "convictions", but so far the Soviet establishment has been content to expel them from professional employment. The new regulations came into force on 1 January 1976, by which time Paritskii, Soifer and Fuks-Rabinovich had presumably all obtained their Candidates' degrees. Until now there had been no suggestion that the regulation should be made retroactive.

From a practical point of view, the loss of their Candidates' degrees should make no difference to the three scientists. Indeed, the change could even benefit them, since several refusenik scientists have been unable to keep menial jobs which they took to try and support themselves, finding themselves sacked because they were "over-qualified", some facing charges of "parasitism" (being without visible means of support). However, from the point of view of Soviet academic life at large, their deprivation sets what could be a dangerous precedent.

Vera Rich

Space economics

Ariane gets an edge

Washington

The fledgling European space industry has scored its first success in the American market. Southern Pacific Communications (SPC), a San Francisco company, has selected the European Space Agency's Ariane rocket for launching its first telecommunications satellites in preference to the Delta launcher of the National Aeronautics and Space Administration (NASA).

Two satellites will be launched for SPC in 1984, which will rent out transponders on the satellites to cable television companies. The deal is a boost for Arianespace, the French-based company which will take over responsibility for the commercial operations of Ariane when the test flights have been completed.

SPC is the first US company to make a firm agreement to use Ariane, although according to Arianespace officials reservations have been made by Western Union, RCA and Satellite Television Corporation and preliminary negotiations have started with GTE. Intelsat, the international telecommunications organization, has signed up for Ariane launches for three of its next series of satellites, due to be placed in orbit next year.

The major attraction seems to be the price. Arianespace is offering extremely favourable terms for a launch, including a requirement that only 20 per cent of the launch price — thought to be about \$40 million for the SPC satellites — need be paid before the launch, with the remainder spread over the following five years.

Officials with the Grumman Corporation, which is acting as the marketing agent for Arianespace, are also making the most of continuing delays that are expected in the initial schedule for launches by NASA's space shuttle. An advertisement placed in the US press shortly after the successful third launch of Ariane from French Guiana in late June lists among the advantages of conventional launchers "well known technology and firm dates for operational customers".

The deal with SPC reflects the growing problems that NASA faces in putting the space shuttle into operational service. Already budget restrictions and various technical difficulties have forced the agency to cut the number of flights planned in the first five years of operation from 48 to 34. Priority in scheduling these early flights is being given to the Department of Defense, requiring several commercial companies either to delay their launches or seek more conventional launches.

Intelsat, which had initially planned to launch its next series of satellites from the shuttle, chose Ariane rather than the Atlas Centaur launcher primarily because of cost. Now SPC, which is eager to tap the rapidly burgeoning field of cable television — and has already sold all the transponders on its first satellite — has selected Ariane also on

cost grounds.

NASA, not surprisingly, is not too happy with these developments. Although accepting what it sees as fair competition from the European space industry, some officials in the agency feel that the generous financing terms being offered represent a government-backed subsidy which they are unable to provide.

But the agency has enough problems of its own to worry about in getting the shuttle into full operation. There is now talk of projected cost over-runs in the shuttle programme next year of up to \$500 million, which could have a significant impact on several of NASA's activities.

One way of absorbing some of these costs, if the Administration does not come up with additional funds, would be to make still further reductions in the initial launch schedule, cuts which would again fall most heavily on commercial customers. Another would be to make deeper cuts in the space science programme, perhaps delaying or even cancelling the Galileo mission to Jupiter, scheduled for 1986.

With the White House talking of further substantial reductions in public expenditure next year, NASA's prospects look bleak. None of this augurs well for the International Solar Polar Mission (ISPM), planned jointly with the European Space Agency. Congress is still debating whether to replace funds for the spacecraft which the United States is contributing to the two-vehicle mission, which was axed by the Reagan Administration in March.

Publicly NASA officials are continuing to express enthusiasm about maintaining the US involvement in the mission. But internal budget proposals sent up to NASA administrator James Beggs for the next year are said not to include any funds for the ISPM spacecraft (nor for a mission to Halley's comet, now being pushed as a sample-return mission to distinguish it from the European Space Agency's Giotto programme). This could change before the final budget proposals are agreed to either by the NASA hierarchy or with the Office of Management and Budget; but nobody is being too optimistic.

David Dickson

●Judy Redfearn adds:

Arianespace says that potential customers have been heartened by the success of the test flight from Kourou, French Guiana, in June. Those already holding launch reservations are apparently beginning to negotiate term contracts. One official guesses that three of the 14 reservations now on the books will be made firm by the end of December. The Ariane rocket will put a satellite into geostationary orbit for between \$27 and \$28 million. Thor Delta launches now cost up to \$7 million more. The attractions of the shuttle, originally estimated to cost between \$15 and \$16 million per launch, have diminished with the uncertainty about the project and the threat that the cost will increase by more than half in 1985 and perhaps by three times that.

Deep sea research

Watery grave?

Washington

In a compromise designed to meet conflicting pressures on oceanographic research, the National Science Foundation (NSF) has decided to incorporate its current Deep Sea Drilling Project (DSDP) into its new Ocean Margin Drilling Program (OMDP), the latter to be financed jointly with the oil industry using the drilling ship *Glomar Explorer* to investigate the geology of the ocean bed.

The decision will mean phasing out *Glomar Challenger*, the ship from which DSDP currently operates. Several scientific goals of the programmes will continue in drilling from the *Explorer*. To accommodate them, part of *Explorer*'s own research programme, to be directed at the ocean margins beyond the edge of the continental shelf, will be delayed.

NSF officials hope that by combining the two programmes they will significantly reduce the total costs of scientific ocean drilling programmes and provide a "realistic framework" within which both can be pursued. In addition to the financial advantages, they concede that the decision should reduce some of the friction that had been caused by the scientific community's concern that commercial goals would squeeze out scientific objectives.

Ten oil companies have so far agreed in principle to contribute towards the costs of the Ocean Margin Drilling Program, announced last year by NSF as a jointly-funded venture (*Nature* 283, 321; 1980). The project had been enthusiastically promoted by the Office of Science and Technology Policy as a model for future collaboration between the public and the private sector.

Many scientists, however, feared that too much of the OMDP activity would be concentrated on areas of the ocean floor of greater interest to the oil industry than to the academic community; also that the series of experiments currently being conducted from *Glomar Challenger*, which can drill holes more cheaply and quickly but is more restricted in its performance, than *Glomar Explorer*, would be brought to a premature conclusion.

Dr John Slaughter, director of NSF, has now announced a compromise. *Glomar Challenger* will be retired in about 1983, when experiments would be transferred to *Explorer*. Plans to equip *Explorer* with a "riser" — a casing around the drill used to prevent blow-outs from occurring if pockets of oil or gas are hit — would be carried out 2 or 3 years later. After that the *Explorer* would divide its time between OMDP (to which industry will contribute about \$18 million a year) and an extension of the current non-riser drilling being carried out by *Challenger*, a solution which seems acceptable to both scientists and industry representatives. **David Dickson**

A Press Council adjudication

This is the text of an adjudication issued by the UK Press Council on 26 August:

The Press Council finds the author was invited to submit an article but not that there was an undertaking that the article would be published. Notwithstanding the invitation, whether it would be published remained a matter for the editor's decision. Alterations which met the criteria laid down by the editor had not been made by the time he told Dr Miller he had decided after all not to publish the article and suggested he send it elsewhere.

The editor did, as Dr Miller complains, submit the article to another scientist mentioned in it, but the Press Council is satisfied he did so with Dr Miller's consent and no complaint can be made at this.

The council is not satisfied the editor used material from the unpublished article in his editorial comment dealing with the same subject. The council, however, believes that the timing of the editorial comment so soon after the rejection of the article was unfortunate. It shares the view put to it by the editor that he ought to have given Dr Miller a fuller answer sooner — and thus an opportunity to submit his article to another scientific journal for consideration before publication of *Nature*'s editorial on the subject. The complaint against *Nature* is not upheld.

The explanatory statement issued by the UK Press Council is as follows:

Although a scientist was invited to submit a magazine article, the decision whether to publish it was for the editor, the Press Council has ruled.

Soon after an invited article was rejected, the magazine commented editorially on the same subject. Despite the unfortunate timing, the Press Council said, it was not satisfied the editor used material from the submitted article.

The council did not uphold a complaint against *Nature* by Dr Jeffrey H. Miller, professor in the Department of Molecular Biology at the University of Geneva that, having refused to publish an invited article submitted to him in confidence and amended by the author according to requirements stipulated by the editor, the editor submitted the article to a person mentioned in it and used some of the material in the article as the basis of his own editorial comment.

Dr Miller suggested an article to *Nature* and was asked to submit it. In it he forecast growing controversy over mixed industrial and academic research into applied microbiology. The article contained criticisms of Professor Charles Weissmann, of the University of Zurich.

Mr John Maddox, who then became editor of *Nature*, sent Dr Miller an amended copy of the manuscript, saying charges against individual scientists should be justifiable and temperate. Dr Miller sent a second version including

many suggested changes but which was still critical of Professor Weissmann.

The editor phoned Dr Miller saying he was ready to publish the article but would like to show it to Professor Weissmann. Dr Miller gave permission. Mr Maddox later said he could not publish the article: it was too directly concerned with Professor Weissmann's work.

Dr Miller then sent a third version, saying he had agreed to the professor seeing the article because he assumed it was agreed in principle to publish it. Although he asked for an explanation if the article was not published, he did not receive one.

Soon afterwards *Nature* commented editorially on the propriety of academics being involved with commercial interests, saying attempts to exploit new genetic manipulation techniques could sour the atmosphere of academic research.

After the solicitors for Dr Miller complained to the Press Council, the editor said it was a convention of the scientific press that personal criticism by outside contributors was not published without warning. He would have replied to Dr Miller if the latter had not told a colleague that he would complain to the council if his third draft were not published.

Dr Miller said the editor's plagiarism of themes not before similarly expressed made it difficult to publish his article elsewhere. Mr Maddox argued that Dr Miller's comment covered the whole academic community while the leading article dealt primarily with molecular biology.

Mr Maddox said there were incorrect and unpubbable charges against Professor Weissmann in the first version. He had had two long phone conversations with Dr Miller. In the second he said he would publish the article if the professor could see it. Discovering that Professor Weissmann acted with propriety had undermined his confidence in Dr Miller's reporting.

Dr Miller commented he had understood the article was accepted for publication and only the Weissmann references were in doubt. The reasons given for withdrawal had no substance and the editor drew heavily on his article.

Dr Miller could not attend an oral inquiry. Mr Maddox told the complaints committee he showed the second version to Professor Weissmann, who explained he had a formal agreement regulating his commercial activities. This was not mentioned in Dr Miller's drafts. Mr Maddox said that his reaction to the third draft was that Dr Miller could have made the amendments earlier but that on reflection he regretted his rather hot-tempered reaction that there was no point in further correspondence.

***Nature* has no further comment to make except to hope that Dr Miller (and Professor Weissmann) will continue to contribute to its columns.**

CORRESPONDENCE

Coping with cuts

SIR — The sad plight of UK universities facing increasing costs and diminishing financial support, possibly even the elimination or demotion of "redundant" universities, moves me to suggest an arrangement not uncommon in American universities, whereby members of university faculties have 9-month rather than 12-month appointments.

This means that faculty members have 3 months in the year free for other activities. Scientists and engineers can frequently find summer employment in government and industrial laboratories, where the broader experience may well be beneficial to their university teaching and research. In any case, the experience may be valuable in combating the ivory tower outlook and maintaining contact with the real world. Colleagues in the humanities may find it more difficult to obtain alternative summer employment, but they would be free to write books, paint pictures, compose music and thereby practice the arts they teach.

In many cases, it may not be necessary for the "9-month scientist" to forsake his laboratory. A large part of American (meaning US) university research is funded by grants from government agencies and from industries. It is common practice for a research proposal to include not only support for a post-doc associate or a graduate student assistant, but also an item for 50 per cent or even 100 per cent of the time of a faculty member for two or three months of the year. Such support is by no means automatic but depends on the quality of the proposal, that is, on the quality of the person making the proposal.

Thus in one way or another the really capable people on university staffs can add usefully to their salaries and those who have "retired" from active productive scholarly work and have little or no standing in their subjects are left where they belong, at a lower level on the financial ladder.

Much good can come from the operation of the free enterprise system, and those who prefer the old, easy-going ways must accept lower annual remunerations.

G.W. BRINDLEY

Pennsylvania State University,
Pennsylvania, USA

Nuclear electricity

SIR — In commenting on the analysis of relative costs of nuclear and coal-fired electricity given in Appendix 3 of the Central Electricity Generating Board (CEGB) 1979/80 Annual Report, I stated that "the full calculation (of costs corrected for inflation) can only be undertaken when the CEGB decides that its present policy of withholding the data . . . is counterproductive" (*Nature* 287, 674; 23 October 1980). I had first asked in specific detail for the data at the beginning of September 1980 and had pursued the matter through both the Department of Energy and the CEGB to the extent of getting a discussion meeting with both department and board officials present. In spite of this the detailed figures of capital cost only became available at the end of March 1981 and then only as the result of a parliamentary question put down on 2 February.

The results of the present calculations, utilizing the newly available data are summarized in the table below. These calculations are significantly different in method from those used in an earlier paper (*Energy Policy*, December 1980, p.344–6) and referred to in my letter of 23 October. The retail price index (RPI) has been used to correct for inflation and a uniform interest rate of 5 per cent has been applied instead of the historic National Loans Fund rate.

However, as the table below shows, even with these modifications the inflation corrected cost of nuclear electricity from Magnox stations is still 18 per cent above that of comparable coal fired stations, instead of 17 per cent below as given by historic costs, and if a previously unconsidered effect of inflation on nuclear fuel costs is included, the margin becomes 34 per cent above that for coal.

Calculations similar to those for the stations of Table 1, Appendix 3 have been done for Hinkley Point B and Drax (first half) (Table 2). The results are also given below. The full paper giving the details of the calculations and dealing also with stations under construction and future stations (Tables 3 & 4, Appendix 3) is in preparation (to be submitted to *Energy Policy*).

Generation costs at nuclear and conventional stations (pence per kWh 1979/80 prices)

Stations commissioned between 1965 and 1979			
	1	2	3
Nuclear	1.30	2.06	2.34
Coal-fired	1.56	1.75	1.75
Nuclear/Coal %	83	118	134

Hinkley Pt B (AGR) and Drax (first half) (coal-fired)				
	4	5	6	7
Hinkley Pt B (nuclear)	1.35	1.80	2.06	2.38
Drax (first half) (coal-fired)	1.52	1.69	1.69	1.69
Nuclear/Coal %	89	107	122	141

Notes to columns: (1) Historic costs as given by CEGB in Table 1, Appendix 3, 1979/80 Annual Report. (2) RPI corrected capital costs, 5 per cent interest rate and fixed costs corrected for load factor equal to availability. (3) As 2, but with the addition of an estimated minimum inflation correction for nuclear fuel costs. (4) Historic costs as given by CEGB in Table 2, Appendix 3, 1979/80 Annual Report. (5) RPI corrected capital costs and 5 per cent interest rate. (6) As 5, but with the addition of an estimated minimum inflation correction for nuclear fuel costs. (7) As 6, but estimated probable fuel cost correction.

Finally may I take up one point in Dr Jones's letter (*Nature* 288, 638; 1980) in which he criticizes my use of the combined coal and oil fuel costs in Fig. 1 of my letter of 23 October. Separate coal fuel costs in p/kWh similar to the combined ones of Table 9 of the CEGB Statistical Yearbook are not available, but figures of coal prices to the CEGB in p/GJ from 1960/61 to 1979/80 have been supplied by the Commercial Controller. When the real price index is normalized to equality with the coal and oil figures for 1972/73 and plotted on the same graph there is no significant difference between the points. I understand from Dr Jones that he was considering National Coal Board production cost data which may well give a different picture, but it is the price to CEGB and specifically the coal fuel cost in p/kWh which I was dealing with and my graph is an accurate reflection of this.

J.W. JEFFERY

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Malet Street, London WC1, UK

Attack on Tamuz

SIR — Your recent editorial "Making Israel atone for Tamuz" (*Nature* 18 June, p.523) leaves out many facts which are important in considering the reasons for Israel's attack and the reality of the situation.

As you and I are aware, nations do what is in their best interest regardless of what treaties they do or do not sign. Just because one nation is a signatory of the Non-Proliferation Treaty does not mean that it will not produce a nuclear weapon. Iraq has been one of Israel's most aggressive and belligerent neighbours, and in fact these two countries are still at war with one another, as no peace treaty has ever been signed. What could this or any other country do if indeed Iraq or any other nation had a nuclear weapon and threatened to use it against an enemy? We could not stop the Soviet Union from invading Afghanistan, nor prevent the problems of Iran. We cannot even clear up the fighting in Lebanon. The world needs Iraq's oil, so "international pressure" would never work.

I agree with your statement concerning the use of oil to generate hydroelectric power, but why did Iraq refuse to accept lower grade uranium that would work very well in a nuclear reactor to produce electricity, but would not be able to produce atomic weapons? Iraq demanded from France weapons grade uranium or their oil supply treaty would be null and void. In addition, one of the inspectors from the International Atomic Energy Agency stated during an interview that the reactor at Tamuz could easily have produced nuclear weapons and that such production could have been shielded from any inspector, thus making the whole question of on-site inspection ludicrous. Although Iraq was supposed to return the spent uranium to France for reprocessing, Iraq also had uranium already purchased from Third World nations, thereby bypassing France completely. You also do not mention that Iraq had contracted with Italy to build a "hot cell" plant that would also allow for enriching and processing plutonium for no other purpose than to build an atomic bomb.

M.S. PASCAL

Englewood, New Jersey, USA

SIR — Israel's attack on the Iraqi reactor may have been politically unwise, even immoral (although I suspect Benthamites might be able to generate some fairly strong Utilitarian arguments in support of the raid). It is, however, something approaching hyperbole to characterize the raid as a flagrant violation of international law (*Nature* 18 June, p.523), as Iraq remains in a technical state of war with Israel, and has repeatedly called for the destruction of the "Zionist entity".

It is also somewhat naive, to be gentle, to assert that Iraq's acceptance of the principles of the Non-Proliferation Treaty assures compliance with its provisions. Soviet citizens who seek the enforcement of the principles of the Helsinki accord often must pack for very cold climates on very short notice.

On judgement day when we must all atone, there may be many whose need for absolution will be greater than that of the Israelis.

ELLIOTT B. GROSSBARD

New York, USA

Neutron bombs

SIR — Neutron weapons, I was astounded to read in your journal, "are a blessing and not a curse". With blessings like that, who needs curses?

That surely is the point about the theory of nuclear deterrence. Its insane logic presents every new curious twist of the nuclear arms race as a blessing. The 50 and 100 megaton weapons were supposed to be a blessing because politicians would hesitate more before unleashing Armageddon. You now argue that neutron bombs are a blessing because they obviate the need to use the big bombs first. Next you will argue, if a low-radiation high-blast bomb is developed, that this is also a blessing since it will destroy property but not people. And a Doomsday bomb that would blow the world asunder would also be a blessing because of the fear of starting a war that it would put in the enemy.

In the last analysis, those who support the nuclear deterrent theory will admit that these weapons are all a curse. That's why one wants nuclear disarmament. But that sane admission cannot co-exist with the dogma that new weapons of death are a blessing. If you allow it to, you become guilty of a form of double-think which is scientifically dishonest and politically and militarily disastrous.

As for the "cynical" (as you call it) argument that neutron weapons will help disarmament negotiations, it is not so much unrealistic, as you argue, as just plain stupid. It is surely obvious that balanced mutual force reductions are more difficult to obtain agreement over the more numerous become the weapons systems deployed on both sides. To argue the opposite is perverse.

I am a "lay" reader of *Nature*. Your support of neutron weapons seems to me to be a betrayal of the efforts of your contributors to understand — and improve — the world we live in. Your arguments do disservice to the entire scientific community and the human values that give it purpose.

MARTIN RABSTEIN

London N5, UK

THE phrase complained of appears in the following sentence: "In the bizarre logic of the nuclear battlefield, in which strategic nuclear weapons are intended to stay forever in wonderland, neutron weapons are a blessing and not a curse." — Editor, *Nature*.

Shielding tanks

SIR — The United States Government and the British Ministry of Defence claim that the neutron bomb is a weapon developed for use against tanks. You have stated (*Nature* 13 August, p.571) that these weapons are more easily (than "ordinary" nuclear weapons) directed against military personnel.

By making reasonable assumptions about neutron weapons concerning the neutron energy spectrum and the ratio of the neutron and gamma energy fluxes it can be shown that a radiation shield could be incorporated into the armour of a tank which would reduce the radiation dose to the crew by a factor of over 100. Such a shield would negate the enhanced radiation properties of a neutron weapon. The neutron bomb would become no more effective than normal nuclear weapons of similar explosive power.

The precise constituents of such a shield would depend on whether it would be attached on the outside of the tank or added as a lining

to the crew compartment. Let us assume that for the tank armour possibly 5 cm of steel can be used as the outer component of the shield. A lining of 10 cm of polythene or other hydrogenous material would thermalize the neutrons and a millimetre of boron or other neutron absorber would remove the neutrons. The inside layer would be a gamma shield and 1 cm of lead would be adequate. Such a shield would add about 5 tons to the weight of a 30 ton tank.

This type of shield, which could be widely incorporated into tanks and military vehicles, would make neutron bombs no more effective against tanks than the small fission nuclear weapons which are reputed to be widely distributed to United States forces throughout the world.

The United States insistence that neutron bombs are for use against tanks in the face of widely available information which enables tanks to be protected against the special effects of neutron bombs, forces the impartial observer to attribute the real reason for the production of neutron bombs to their potent ability to kill people whilst causing only the minimum of collateral damage.

J.E.F. BARUCH

The University,
Leeds, UK

Local reaction

SIR — Apparently, Lord Rutherford's maxim that there is mathematics and physics and that all other activities are stamp collecting, is still valid in Ms Rich's writings on Yugoslav scientific institutes. The result is a biased assessment of the scope of their competence, specifically in problems related to nuclear power (*Nature* 11 June, p.446–447). A quick check with the *Science Citation Index* would show the scientific activities of the two cited "physics" institutes to cover a broad range in Rutherford's "philatelic" category.

Contrary to the statements in *Nature's* article, there is no "local misunderstanding of the distinction between the charging of a reactor and its start-up". There is no misunderstanding or lack of knowledge with respect to reactor or nuclear fuel technology, much less on the importance of additional power sources in a power-hungry country with few significant energy resources still available for exploitation. Nor has there ever been any misunderstanding based on ideological doctrinarism, contrary to what could have been perceived from another article by the same author (*Nature* 288, 5; 1980).

The dispute is over the technological and organizational discipline necessary in building, starting and operating high technology units like nuclear reactors. The one mishap, in 1958, was the result of just such a typical breach of work discipline.

The dispute is rather between the narrow, mission-oriented technocrats and the concerned scientists on the consequences of siting large facilities in an already ecologically strained region. Scientists insist on careful planning and broadly based environmental impact assessment. Their concern reaches beyond the narrow, albeit possibly correct, advice of "no technological obstacles", offered by a reputable international expert to our supposedly less developed country. The dispute is well within the framework of questions raised in reviewing the Final Safety Analysis Report, and concerns another problem — that of the reference plant for the

Krško unit.

The scientific community is not beyond reproach either. Its lack of credibility, or "weight", with the decision-making social strata stems from some recent and not so recent failings. But it should not be blamed for either disciplinary monoculture or primitive ecological extremism.

I could not refrain from voicing my displeasure with these articles, although this letter might prove just another exercise in futility. Ms Rich's articles remind me of a statement made by one of your great historians. Writing about an infamous propaganda minister of recent European vintage, he claimed that the points in the minister's propaganda items were made so well, that even the opposite of it was still not the truth.

VELIMIR PRAVDIC

Ruder Bošković Institute,
Zagreb, Yugoslavia

VERA RICH WRITES — I cannot accept Dr Pravdic's statement that there is no "local misunderstanding". During my visit to Zagreb in March and April of this year, I spoke with a number of members of the public who interpreted the announcement of the imminent "charging" of the reactor to mean that it would "start working" immediately. I fully agree that the main dispute lies between the "concerned scientists" and the technocrats. Perhaps the undoubted local apprehension arises from a fear that in such a conflict, the technocrats may win.

Mystery genre

SIR — In reading Edmund Leach's review of the book *Genes, Mind and Culture* by C.J. Lumsden and E.O. Wilson (*Nature* May 21, p.276) it strikes me that comments such as "crass idiocy", "parody of science", "gibberish" and "phoney" might be a trifle intemperate coming from a reviewer who admits that he cannot comment on the bulk of the work, which is in neuroscience and psychology.

What is far more intriguing is the question of what Leach could have had in mind when he referred to the "genre" to which *Genes, Mind and Culture* belongs. At first it went right by me, but I did a double take later when I recalled that Leach had once written a review on the genre of "popular ethology" books, such as those of Lorenz and Ardrey. It was hilariously entitled "Don't say 'Boo' to a Goose" and appeared in the *New York Review of Books*.

That was 15 years ago. Is it possible that Leach is still referring to the same genre? I ask, because — significantly — he does not mention the major theoretical work *Sociobiology* in his list of E.O. Wilson's credentials. Worse, he calls Wilson a "popularizer". Surely there is not a regular reader of *Nature* anywhere — on either side of the well-known floor — who conjures up the image of "popularizer" when Wilson comes to mind.

I urge that Leach be invited to identify the mysterious genre in which he has classified the Lumsden–Wilson book and if it is ethology — there is virtually no ethology in *Genes, Mind and Culture* — then clearly his review must be rescinded from the pages of *Nature* as having been written by a uniquely unqualified party.

N. JACKSON

Belmont,
Massachusetts, USA

Nobel Physics Prize in perspective

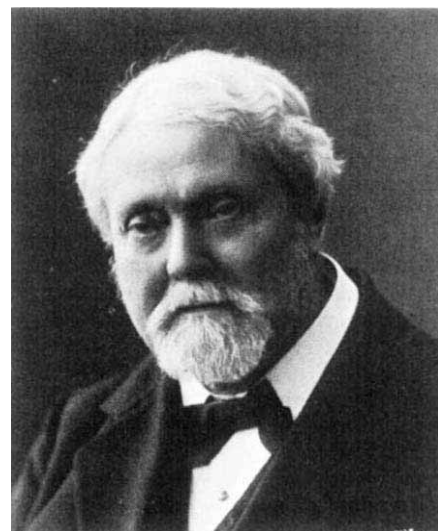
Robert Marc Friedman*

WITH the time for the announcement of the 1981 Nobel Prizes approaching, it is timely to consider the nature of these awards. There is a need for a wider understanding of the process by which these honours are determined: a realistic appreciation of the prizes can serve only to enhance their value¹. So far, however, little has been written about the decision making process. By drawing on historical research, I shall seek to elucidate some of the issues which have been raised, especially relating to the scope of the prize in physics.

To what extent does Nobel's will define the field of physics that can be considered for a prize? Little was specified in Nobel's will and, while drawing up the statutes, the prize-awarding institutions resisted all attempts to delineate or define the broad terms "physics", "chemistry" and "physiology and medicine" used in the will². Hence, when the members of the committee for the prize in physics met for the first time in 1901, they were faced with

B. Hasselberg, wrote that the prize could be awarded for great achievements not only in "pure Physics properly so-called", but also in "the sciences most closely connected with Physics and for the cultivation of which physical methods are employed[;] as [such] Astrophysics and physical chemistry are also to be taken in account. This I suppose will certainly answer to the proper meaning of the testator. Whereas thus . . . pure Astronomy or celestial dynamics or Astrometric [sic] will scarcely be included, any work on Astrophysics of great importance deserves certainly a thorough consideration"³.

Moreover, the inclusion of meteorology raised little, if any, objection; as well as acting as host for this meeting, the Meteorological Institute's director, Professor H. H. Hildebrandsson, served on the original committee. At the meeting, committee members called for strengthening the expertise in "cosmical physics" (meaning



H. H. Hildebrandsson, professor of meteorology and member of the committee for physics (1901–1910), hosted the committee's first meeting.

statutes, cannot be divorced from the development of the physics discipline in Sweden. Consequently, various traditions have been established for interpreting the statutes, which arose from past conflicts, debates and strategies for attaining authority, that have little relevance for the present. Traditions grounded in past contingencies ought not be mistaken for immutable, formal rules and regulations. Unfortunately, literature concerning the Nobel Prizes tends to discuss the awards in the passive voice, and thereby tends to reinforce the mystique of anonymity and objectivity in the decision process. This frequent use of the passive voice also slights the committee members, the difficulty of whose task is easily overlooked.

The history of the award of the Nobel Prize in Physics, which is partly the history of interpreting the statutes, also depends on the development of physics in Sweden. The now traditional restricted scope of the prize has its origin in past controversies, not formal rules. Einstein's relativity and Bjerknes's meteorology were both casualties.

the same ambiguities in the statutes that we recognize today. How ought they interpret and reconcile such clauses as "have conferred the greatest benefit on mankind" and "most important discovery and invention within the field of physics"? The provision in the will that works "during the previous year" should be interpreted to mean "for the most recent achievements . . . and for older works if their significance has not become apparent until recently", created further opportunity for uncertainty and arbitrariness.

During the early years, both the committee and the executors of the estate understood that physics ought to be defined as broadly as possible³. At the first committee meeting, held at the Meteorological Institute of the University of Uppsala, the committee agreed that astrophysics was eligible while astronomy was not⁴. More specifically, in responding to an inquiry, the head of the committee,

the physics of the macro-world) on the grounds that nominations from this branch of physics would surely increase⁶. In fact, during the next thirty years, nominations to honour various astrophysicists, geophysicists and meteorologists were not and could not have been formally disqualified for being outside "physics" (for example, G. E. Hale, H. Deslandres, K. Birkeland, C. Størmer, N. Shaw, J. Hann, V. Bjerknes). In short, neither Nobel's will nor the statutes restricted the definition of physics to a narrowly defined group of specialties.

Narrow scope

I shall argue that the subsequent restriction of the scope of the physics prize stemmed from a determined effort within the Swedish physics discipline. Not surprisingly, Nobel Prize deliberations became enmeshed in the process by which various factions within the Swedish physical science community attempted to define and to legitimize their particular notions of physics: its objects, methods and goals. The history of prize decisions, being also the history of interpreting the

The Nobel Committee

Committee members play critical roles by evaluating nominees and selecting nominators. During the period for which committee archives are accessible (1901–30), the committee's five members judged annually the nominations received and then proposed to the physics section (*Klass*) of the Royal Swedish Academy of Sciences how the prize should be allocated. Following approval from the *Klass*, or suggestion of an alternative proposal, the full Academy then voted on the award. On some occasions, the *Klass*, and even the Academy, disagreed strongly with the original choice, so that the committee had to justify its actions carefully, especially when these were controversial or not unanimous. The committee members also drew up each year a list of institutions and individuals who would be invited to send nominations for the following year. Thus the committee regulated to some extent the nominators who might supplement the fixed group entitled to make nominations — members of the Academy, former prize winners, and professors of physics at Scandinavian universities and technical colleges.

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Dissent over Einstein

The influence of the Swedish physics discipline on Nobel Prize decisions is well illustrated by Albert Einstein's prize. In 1922 the Academy voted to award Albert Einstein the previously "reserved" 1921 Nobel Prize "for his services to theoretical physics, and especially for his discovery of the law of the photoelectric effect". Both the timing and the citation have long raised questions among Nobel Prize watchers. If the opinions of the scientific community at large, or more specifically of the nominators, had been decisive, Einstein would certainly have received a prize earlier, and for his theories on special and general relativity.

As early as 1910, W. Ostwald began nominating Einstein for his work in special relativity. During the First World War and immediately thereafter, many nominations for Einstein arrived specifying his research on relativity, brownian motion and specific heats of solids and/or quantum theory in general. Following the famous 1919 solar eclipse experiment which, according to many, confirmed Einstein's prediction of the bending of light by the gravitation of a massive body, a virtual flood of nominations for Einstein ensued.

Yet, in 1920, the committee and the Academy agreed to award the prize to Charles Guillaume. One year later, claiming that they could not find any grounds for awarding a prize to Einstein, the committee, followed by the Academy, voted to reserve the 1921 prize⁷. Finally, in 1922 Einstein received a prize, specifically for the law of the photoelectric effect — not for his quantum theory to explain the law, nor for relativity in any form. Of the approximately fifty nominations of



W. Ostwald nominated Einstein for the 1910 physics prize, specifying work on "the relativity principle". By 1922 many physicists had proposed awarding a Nobel Prize for Einstein's relativity theories; these included: Bohr, Eddington, Haas, v. Laue, Lorentz, Planck, Sommerfeld and Zeeman.

EINSTEIN'S RELATIVITY THEORY OF GRAVITATION

The debate on Einstein's theory was in full flow in *Nature* of 4 December 1919. E. Cunningham wrote:

THE results of the Solar Eclipse Expeditions announced at the joint meeting of the Royal Society and Royal Astronomical Society on November 6 brought for the first time to the notice of the general public the consummation of Einstein's new theory of gravitation. The theory was already in being before the war; it is one of the few pieces of pure scientific knowledge which have not been set aside in the emergency; preparations for this expedition were in progress before the war had ceased.

The question from which Einstein began the great advance now consummated in success was this. If energy and inertia are inseparable, may not gravitation, too, be rooted in energy? If the energy in a beam of light has momentum, may it not also have weight?

The mere thought was revolutionary, crude though it be. He asked for an out-and-out relativity of space and time. He would have it that there is not ultimate criterion of the equality of space intervals or time intervals, save complete coincidence. All that is asked is

that the order in which an observer perceives occurrences to happen and objects to be arranged shall not be disturbed.

This, then, was the mathematical problem. The pure mathematics required was already in existence. An absolute differential calculus, the theory of differential invariants, was already known. In pages of pure mathematics that the majority must always take as read, Riemann, Christoffel, Ricci, and Levi-Civita supplied him with the necessary machinery. It remained out of their equations and expressions to select some which had the nearest kinship to those of mathematical physics and to see what could be done with them.

Although in the letter immediately before Cunningham's report, Alexander Anderson of University College Galway concluded:

I THINK it is quite likely that if the refraction of the atmosphere of the Earth due to density changes during an eclipse could be accurately obtained and allowed for, it would be found that there is no Einstein effect at all.

Einstein over the years, the law of the photoelectric effect was specified by only one nominator. Certainly, a familiarity with the committee must be sought to begin understanding these events.

Experimental bias

At the time, three of the committee's five members belonged to the Uppsala tradition of experimental physics — B. Hasselberg, G. Granqvist, and A. Gullstrand. The others were S. Arrhenius (physical chemistry) and V. Carlheim-Gyllensköld (mathematical and cosmical physics). The committee's strong experimentalist bias proved significant from the start in determining Nobel Prize decisions and for interpreting the statutes.

Debates on the role of mathematics and theory in physics that had begun in the 1890s carried over into deliberations on the prize. Hence, even in the case of a worldwide campaign for Henri Poincaré, in which his mathematical physics *qua* physics was delineated from his purely mathematical accomplishments, the committee did not see fit to recommend him for the prize⁸. In a protest note, Carlheim-Gyllensköld pointed out the committee's general unwillingness and inability during the first ten years to evaluate nominations in mathematical physics such as those of Boltzmann, Heaviside, Kelvin and Poynting⁹. Arrhenius had commented that Uppsala physicists consider "spectral analysis . . . the only part of physics worth pursuing"¹⁰. Indeed, Hasselberg did "all in my power to procure the prize" (1907) for A. A. Michelson, who had received but two nominations, not for his role in the aether-drift experiment but, rather, primarily for his work in precision spectroscopy and metrology¹¹. Hasselberg admitted that he found a "sympathy for an

area closely connected with my own speciality . . . I cannot but prefer works of high precision"¹².

Other examples abound. Thus the proposal for the 1908 prize was sabotaged partly by those who believed it "unjust" to award a prize to a theoretician, M. Planck, without dividing the honour with an experimentalist¹³. Apparently, to ensure the defeat of the Planck nomination, the Academy was informed of Planck's questionable "hypothetical molecules of energy", which were not mentioned in the committee's report¹⁴. Not surprisingly, the majority's stringent demands that theory be completely verified by experience virtually precluded a prize for Einstein's various theoretical endeavours. Yet, did the 1919 eclipse experiment have any impact on the committee's evaluation?

Although often hailed as a "crucial



B. Hasselberg. "I cannot but prefer works of high precision" — on A. A. Michelson's spectroscopic measurements. "It is highly improbable that Nobel considered speculations such as these to be an object for his prizes" — on Einstein's relativity theories.

experiment" that proved Einstein's general relativity, the 1919 test left many reasonable physicists unconvinced, among them the members of the Nobel committee for physics. In 1920 Arrhenius prepared a special report on the subject for the committee in which he noted that objections could be made against the accuracy of the observations, which were therefore not a proof of the prediction¹⁵. In any case the prize for 1920 seemed already determined. Hasselberg then lay gravely ill. Having championed precision measurement, especially in spectroscopy, Hasselberg now favoured his colleague on the International Bureau of Weights and Measures, Guillaume, whose work in metallurgy was of importance for metrology. To express appreciation for Hasselberg's work on the committee since 1901, the Academy could pay homage to him in this manner.

Nevertheless, the Einstein campaign continued to grow in 1921, with strong support for the relativity theories. To ensure a sound evaluation, the committee assigned the task of writing a special report focusing on Einstein's relativity and gravitational theories to its esteemed member Gullstrand, who would have received the 1911 prize in physics for his contributions to dioptrics had he not first been named for a prize in medicine¹⁶. In his fifty-page report, Gullstrand concluded that neither the general nor the special theory of relativity warranted a Nobel Prize¹⁷. In 1922 he brought his report up to date and came to the same conclusion. Acceptance of these theories remained simply "a matter of faith (*trossak*)"¹⁸. He resolved that Einstein must never receive a prize¹⁹.

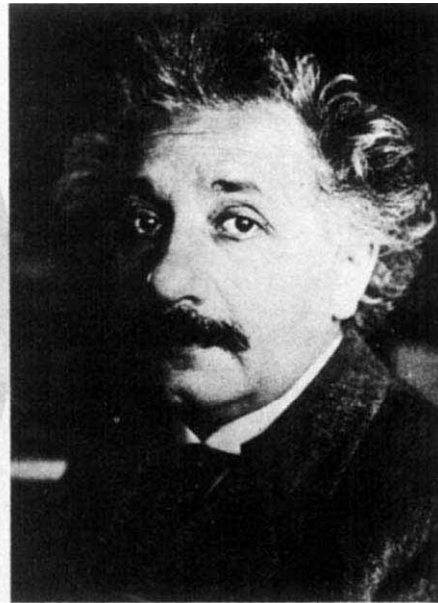
The 1922 Prize

In general the committee supported Gullstrand. Hasselberg asserted from his sickbed that "it is highly improbable that Nobel considered speculations such as these to be an object for his prizes"²⁰. Nor was there much sympathy for honouring Einstein's other theoretical contributions. Even his theory of the photoelectric effect was not considered significant enough to warrant a prize, since much of the elaboration of the quantum theory entailed others' endeavours²¹. Yet, when the *Klass* met to vote on the committee's proposal to reserve the prize, mathematical physicist C. W. Oseen suggested that Einstein's law, rather than the theory, ought to be considered for a prize since this law forms the foundation for all the recent remarkable achievements in atomic physics²². The *Klass* then voted to acknowledge the significance of Einstein's discovery of the law of the photoelectric effect, but resolved that it was not worthy of a prize²².

Oseen did not intend to let the issue end there. He was concerned not so much with Einstein but with Niels Bohr, who had been repeatedly nominated since 1917²³. He regarded Bohr's atomic model as "the



A. Gullstrand (left) prepared special reports on Einstein's relativity and gravitational theories in which he concluded that they did not warrant a Nobel Prize. The acceptance of these theories remained, even after 1919, "a matter of faith (*trossak*)".



most beautiful of all the beautiful" in theoretical physics, and was thus determined that this work should receive a prize²⁴. Moreover, he had already begun on a campaign to strengthen Swedish physics, especially by introducing theoretical physics. For Oseen, atomic physics offered the challenge of theoretical investigation while remaining tightly disciplined by experiment. Previously, the experimentalists on the committee balked at approving Bohr's atom model which, they claimed, stood "in conflict with physical laws" and hence reality²⁵.

Now, in 1922 Oseen proposed Einstein for the discovery of the law, and then joined the committee as a special extra member. Oseen wrote special reports on Einstein's law and on Bohr's atomic theories. He carefully argued that experimental investigations had confirmed Einstein's law so thoroughly that it must be considered among the "soundest propositions physics now possesses"²⁶. Hence Bohr's models, which he argued were built on this law, must be regarded as being in solid agreement with physical reality²⁶. Finally, not without some difficulty, Oseen prevailed. Although further study is needed, this episode demonstrates the importance of the committee membership's interests and philosophies of science for Nobel Prize decisions.

The Einstein deliberation came at a turning point in the development of Swedish physics and of the committee. By 1923, after the deaths of Hasselberg and Granqvist, Oseen was elected to the committee as a regular member and was joined by his Uppsala colleague, atomic physicist Manne Siegbahn. Together with Oseen's friend, Gullstrand, the three Uppsala physicists commanded a majority on the committee and could plan and act together on Nobel Prize matters. Oseen's broad knowledge, international reputation

and aggressive determination allowed him to assume leadership in the committee. His vision called for an overhaul of Swedish physics: stronger links with major foreign research centres, and greater visibility and prestige to ensure adequate research funds and university positions²⁷. By promoting atomic physics, in which experiment and theory intimately progress together, he hoped to overcome traditional prejudices and to institutionalize a theoretical physics standing apart from abstract mathematical physics and mechanics. Yet, to be successful in this "new epoqe"²⁸, which he declared after the election of Siegbahn to the committee, they not only had to promote and to legitimize their preferred research programmes, but would also have to eliminate what they considered insignificant specialities. Starting in the 1920s, the Uppsala group began a determined campaign to restrict the definition of physics within the Academy and with regard to the Nobel Prize. To pursue this strategy they could try to change the statutes formally, control membership in the *Klass* and committee and/or establish traditions for interpreting the statutes by honouring particular specialities and blocking others.

Arrhenius and astrophysics

Change of statutes entails considerable debate and great difficulty, since the entire Academy must vote on such motions. Thus the committee members tried to avoid formal motions to change the statutes since these inevitably breed conflict within the committee and the *Klass*, which in turn weakens their posture in the Academy. This became clear when, in 1923, the new Uppsala group and Arrhenius attempted to eliminate astrophysics from the scope of the prize in physics. Arrhenius, who wrote the proposal, seems to have feared that the establishment of new Nobel institutions



S. Arrhenius, member of the committee for physics, had originally supported cosmical physics, but in 1923 he tried unsuccessfully to eliminate formally astrophysics from the scope of the Nobel Prize for physics.

would threaten his own Nobel Institute for Physical Chemistry. (According to the statutes, Nobel funds can be used to establish research institutes where nominations can be evaluated.) Previously, only Arrhenius's institute had been supported in this way, but during the war Carlheim-Gyllensköld suggested a department for cosmical physics that was later followed by detailed plans for an astrophysics centre^{29,30}.

Consequently, Arrhenius proposed that astrophysics no longer should be considered part of physics. Although previously a strong supporter of cosmical physics, he now argued that astrophysics had progressed so dramatically, that it now incorporated all physical astronomy³¹. Thus, he concluded, astrophysics is astronomy, and therefore not part of physics. Carlheim-Gyllensköld vigorously opposed this action and, predictably, the statute change was defeated³². A much easier strategy entails dismissing outright nominations in cosmical physics as "not being significant for physics", the phrase commonly used by writers of the general report to the Academy. Hence, Hale and Deslandres, previously considered certain winners of the prize, could now be quickly dismissed once Hasselberg had died and Arrhenius no longer supported these sciences.

To proceed in this way requires a committee and a *Klass* that does not object too strongly — so that membership must be controlled when possible. When the physics section of the Academy expanded from 6 to 10 members in 1904, it was given the title "Physics and Meteorology". Having established strong research traditions in meteorology during the nineteenth century, Swedish meteorologists received a number of places in the *Klass*. By 1919, however, the promise of Swedish meteorology had waned and a

long, bitter and inconclusive feud concerning atmospheric thermodynamics raised doubts over the personal and/or scientific reliability of some of the meteorologists. Oseen was prompted to express concern whether any branch of "terrestrial physics" could become rigorous, given the scale of geophysical phenomena and lack of laboratory testing possibilities³³; he sensed a "relativity of all knowledge" within these sciences³³. Uppsala experimental physicists shared this belief. They attempted to reduce the meteorological influence in the *Klass* and prevent the awarding of a Nobel Prize to this science, as this would legitimize meteorology as part of physics in the Academy.

Meteorology out of favour

The campaign against meteorology in the *Klass* seems to have begun after the death of foreign member J. Hann, an Austrian meteorologist. The election of foreign members has a double significance. On occasion, such an election is a stepping stone for prospective Nobel Prize candidates; in general, all foreign members have the right to propose candidates for the prize. In this instance, members of the committee tried to obtain before the meeting the signatures of a majority of the *Klass* for the appointment of a physicist, thereby precluding replacing Hann with another meteorologist³⁴. Arrhenius sketched a nomination for Planck and travelled up to Uppsala; however, rather than speaking with meteorologist Hildebrandsson as promised, he carefully avoided him while meeting the other physicists. The elderly Hildebrandsson felt betrayed³⁵. But his subsequent request at the meeting of the *Klass* that they promise to elect a meteorologist next time, was defeated³⁶. And, when foreign member Röntgen died the following year, Gullstrand organized a comparable campaign against electing a meteorologist, saying he would refuse to vote if a meteorologist were proposed³⁷.

Similarly, when *Klass* members Hasselberg, Granqvist, and Bäcklund died, they were rapidly replaced by physicists Siegbahn, Pleijel, and Oseen. After the death of meteorologist N. Ekholm in 1923, Hildebrandsson feared the worst and decided to take the offensive by bringing the touchy issue into the open and up to the entire Academy. Now that physicists had twice been elected to succeed meteorologists, "should this happen yet again, then it must be regarded as a wish of the physicists to get rid of meteorology within the Third *Klass*"³⁸. He pleaded that a meteorologist should now be elected because, out of the ten members of the *Klass*, the two remaining meteorologists were so old that there was a real danger that meteorology would soon lose its representation in the Academy. To refuse Hildebrandsson would have been a personal insult, so that his candidate, A. Wallén, was elected³⁹. Nevertheless, when

the other meteorologist, Hamberg, died shortly afterwards, Gullstrand warned members of the committee that "under all circumstances we must act [*ställa oss*], so that we do not get in yet another weak meteorologist"⁴⁰.

This time they acted quickly. Led by committee members Oseen, Siegbahn, Gullstrand and Arrhenius, a majority signed a proposal for physicist C. Benedicks. Carlheim-Gyllensköld, a cosmical physicist, opposed these efforts. When he next came up for re-election on the Nobel committee, Oseen, Gullstrand, and Siegbahn tried to remove him. First, they tried to reduce the number of committee members from five to four, thereby eliminating his position. When this tactic failed, they used their right to elect a foreign member — and proposed Niels Bohr. Both committee and *Klass* approved the Oseen-Siegbahn nomination, but the Academy again sensed an Uppsala grab for control, and voted to retain Carlheim-Gyllensköld⁴¹.

Similar tactics were used within the committee to avoid awarding Nobel Prizes for meteorological research. Occasional nominations for a meteorologist were dismissed as "not significant", or "not confirmed by experience"; but, one nominee reappeared repeatedly, with increasing support from both physicists and meteorologists. Oseen and his Uppsala colleagues were determined that he — Vilhelm Bjerknes — should not receive a prize.

The Bjerknes group

Between 1918 and 1924 a group of Norwegian and Swedish scientists working in Bergen (Norway) under the leadership of Bjerknes transformed theoretical and practical meteorology. Their various conceptual achievements included a new model of the extratropical cyclone, the type of low-pressure system common in the mid-latitudes. Claiming that these atmospheric disturbances are composed of three-dimensional surfaces of discontinuity — fronts — they began conceiving of a cyclone as a wave that forms and evolves along pre-existing polar fronts separating polar and tropical air masses. Their theory provided the first clear physical model of cyclonic evolution, which in turn provided for direct interaction between theory and practice.

The Bergen meteorologists constructed their models over a period of time partly as a result of changes in forecasting methods and partly in conjunction with their efforts to forge new forecasting techniques to meet challenges arising from agriculture, aviation and fishery. Earlier, Bjerknes had for almost two decades worked on an ambitious project to "transform the inexact science of meteorology into an exact physics of the atmosphere". As such, theoretical and physical reasoning informed both the forecasting effort and the new concepts⁴². Having been a

professor of *Stockholms högskola* from 1894 to 1907, Bjerknes had various supporters — and opponents — within the Swedish scientific community. In particular, the national rivalry that occurs when Norway outshines her traditionally richer and culturally superior neighbour provoked a general reaction of “humbug” from some Swedish scientists⁴³, making it easier for Oseen and others to block Bjerknes’s candidature in 1923, 1924, 1926, 1929 and repeatedly in the 1930s.

During the 1920s, several reasons were given for excluding Bjerknes. In brief, the general reports by the committee claimed that the various “empirical” discoveries and improvements in forecasting had arisen simply as a consequence of an improved network of observation stations⁴⁴. Moreover, Bjerknes’s baroclinic circulation theorems for fluids in which density is a function of both pressure and temperature (1898, 1903), should not be considered a discovery or invention; according to Oseen, these innovations and their application to atmospheric and oceanic phenomena were already implied in Helmholtz’s earlier circulation theorem for ideal fluids⁴⁵. Moreover, Oseen and Gullstrand repeatedly asserted that the wave-theory of

baroclinic fluids; indeed, several years earlier, he had declared that Helmholtz received too much credit in this area⁴⁸. When L. Silberstein derived these theorems independently of Bjerknes, he dismissed such fluids as pure theoretical constructs. Bjerknes’s achievement was that he recognized their profound implications for the study of atmospheric and oceanic motions. Revealingly, shortly after Oseen and Gullstrand argued that Bjerknes’s work cannot be considered a discovery or invention, they urged the *Klass* to recall that Nobel’s meaning of “discovery and invention” must be interpreted as *broadly* as possible⁴⁹; on that occasion they were defending a candidate for the 1924 prize against objections of the sort they had levelled against Bjerknes, the nominee here being their colleague, Manne Siegbahn.

Uppsala and geophysics

The cavalier manner in which Bjerknes’s work was dismissed was apparent to those who were close at hand. Otto Pettersson, who had sat on the Nobel committee for chemistry and who believed that Bjerknes deserved a prize, recognized the pattern and noted in dismay that apparently, “Uppsala doesn’t consider geophysics to be physics”⁵⁰. He also wondered whether the committee was competent to appreciate Bjerknes’s work or whether Oseen and Gullstrand would ever bother to read enough of it to be able to judge it fairly⁵¹.

Oseen provided other reasons to confirm Pettersson’s fear that scientists with great authority all too often misuse their power⁵². After Hildebrandsson’s death in 1925, Oseen found an opportunity to use meteorologists against Bjerknes. For the election to the *Klass* he and other Uppsala scientists backed F. Åkerblom, an opponent of the Bergen school and of Bjerknes personally. But when a letter from Bjerknes was distributed suggesting that some of Åkerblom’s research results seemed to have been borrowed from others, Åkerblom was dropped, leaving as potential candidates two former Bjerknes assistants, V. W. Ekman and J. W. Sandström⁵³. When Sandström was eventually nominated, Oseen almost exploded with rage claiming he would show that Sandström did not even understand the basic physics behind meteorology and oceanography⁵³. He demanded and received a postponement of several weeks. But instead of demolishing Sandström’s candidature, Oseen and Siegbahn had the original proposal replaced by one of their own. Apparently Oseen had learned that Ekman — the alternative — was planning to campaign for Bjerknes and therefore opposed him in spite of an earlier declaration that Ekman’s work stood clearly above Sandström’s⁵⁴.

Oseen also made use of an attack by the volatile Sandström against his friend Bjerknes, written in 1923 at a moment of great personal despair. Sandström’s harangue followed an interview with

Royal Meteorology Society: Symons Gold Medal

From *Nature* of 6 January 1940:

THE decision of the Council of the Royal Meteorological Society to award the Symons Gold Medal for 1940 to Dr J. Bjerknes will be very popular among British meteorologists, to whom he has become well known during his frequent visits to this country. In 1932 the medal was awarded to his father, Prof. V. Bjerknes, and it is fitting that the son, who shared the work, should also share the honours.

In 1933 the accumulated research of the Norwegian school of meteorologists into the dynamics of the atmosphere was published in book form under the title “*Physikalische Hydrodynamik*” by V. and J. Bjerknes, H. Solberg and T. Bergeron. Dr Bjerknes is still young, and we look forward to further important research in future from these brilliant Norwegians.

Bjerknes carried in the Stockholm daily *Dagens Nyheter* entitled, “Norway, meteorology’s leading nation”⁵⁵. In a published response, Sandström said that the polar front theory has not been effective in Sweden nor elsewhere, and that the wave-theory of cyclones conflicted with empirical experience in Sweden. He added that “Norwegians are almost too vigorous when it comes to creating new hypotheses and theories, and it is neither desirable nor practical that we follow them in all their capers and flights of fancy [*krumsprang och hugskott*] of this sort”⁵⁶. Oseen in his support for Sandström’s election to the *Klass*, claimed therefore that Sandström had during the past few years tested empirically Bjerknes’s theories for the polar front and for cyclones, finding that “the first theory is not confirmed by the meteorological phenomena in Sweden while the Bjerknes equations for cyclones do not stand in agreement with the actual [observed] conditions”⁵⁷. Sandström was elected and Oseen now had a basis for arguing against Bjerknes’s candidature. Yet, by the 1930s, most meteorologists were forecasting with the Bergen school’s methods and working on theoretical problems associated with them. So Oseen and other committee members who preferred a restricted definition of physics had to find another strategy.

By 1936 the case for awarding a prize to Bjerknes and his chief assistants was strong. Ekman, who supported Bjerknes and who was now a member of the *Klass*, requested that the committee prepare a special report on Bjerknes, even though C. D. Anderson was “waiting in turn”⁵⁸. Unable to avoid the issue, Oseen began a detailed report on the Bjerknes school’s activities. Apparently, he concluded that regardless of the value of their work, meteorology could not be considered as part of physics, and hence not in the domain for the prize⁵⁹. Ekman protested, asserting that meteorology and weather forecasting, when based on solid physical



C. W. Oseen. His goal of promoting theoretical physics and of improving the international stature of Swedish physics motivated him to attempt restricting the definition of physics within the Academy and for the Nobel Prize.

cyclones and the polar front theory are not proven by experience⁴⁶. Lastly, they held that the Bergen meteorology had been accepted only by a limited number of scientists⁴⁷. How valid were these objections to the Bjerknes nominations?

First, the claim that the Bergen models and improved forecasting arose simply because of the refinement of the observation network cannot be substantiated. Comparable networks had been introduced during the war without similar fruitful results; moreover the Bergen successes did not all immediately ensue. More telling in intent is Oseen’s claim that Bjerknes’s discovery of the baroclinic circulation theorems did not qualify as a discovery in the sense that Nobel had specified. Yet, as a hydrodynamicist, Oseen knew full well that Helmholtz had never considered the possibility of

principles such as those of Bjerknes, perfectly meets Nobel's intentions of both being significant in physics and benefiting mankind⁶⁰.

Knowing that Oseen dared not try to change the statutes formally to exclude meteorology, Ekman suggested that Oseen should take the matter up before the Academy⁶⁰. Should the committee and the *Klass* believe Bjerknes worthy, they could propose him for a prize and, also, suggest an alternative candidate within Oseen's "restricted definition of physics". Thus, should the Academy decide that meteorology is not eligible, the committee would still have control over the alternative candidate. In short, Ekman suggested that the Bjerknes case could be a precedent for interpreting the statutes⁶⁰. But, Oseen did not want to risk having a precedent for meteorology being part of physics. To ensure that the Bjerknes nomination did not reach the Academy, by having it first rejected in the committee or in the *Klass*, Oseen apparently included in his special report a disparaging personal attack. According to Sandström, who was in the *Klass*, Bjerknes would have probably received a prize had Oseen not written his "perfidious Bjerknes biography"⁶¹. Oseen seems to have chosen Bjerknes's outmoded interests in physics to discredit him as a scientist. Bjerknes had found it impossible to abandon his father's search for analogies between hydrodynamic and electromagnetic fields of force. As a young man Oseen had also worked in this area, but he now considered these methods and goals of the 1890s to be totally absurd and even contemptible⁶². By portraying Bjerknes as a hopelessly plodding

physicist, who neither accepted nor understood modern physics, Oseen tried to convince the committee and *Klass* that Bjerknes would be an embarrassment for the Academy. Oseen related how with "special satisfaction" he could use against Bjerknes the latter's belief that Einstein's relativity theory marked the end of classical physics, rather than the start of the new⁶³. Finally, to finish off the Bjerknes candidature Oseen solicited comments from yet another Bjerknes assistant, who was then attempting to establish a rival school of meteorology in the United States: "For practical weather forecasting . . . Bjerknes's calculations have absolutely no significance whatsoever"⁶⁴. Although effective in preventing Bjerknes from receiving a Nobel Prize, this statement, in this form, is false.

Oseen died in 1942 having successfully blocked the awarding of a Nobel Prize for work in the geophysical sciences. His goal of promoting theoretical physics and of improving the international stature of Swedish physics had led him to attempt restricting the definition of physics within the Academy and for the prize. Yet, there is no formal reason based on Nobel's will for excluding geophysics and astrophysics from the prize. Rather, there is only a tradition of interpreting the statutes that has been informed by past personalities and contingencies. Oseen and other committee members recognized this to be true. When Bjerknes confronted him with solid evidence that Nobel considered meteorology and geophysics to be part of physics, Oseen replied that "the interpretation of words changes from person to person. What do we mean by

physics? What do we mean by 'during the preceeding year' or 'recently'? . . . Do any words exist that have a fixed significance?"⁶⁵. As soon as one finds an interpretation "consistent with one statute, one finds another inconsistent"⁶⁶.

The summing up

Inevitably, the interpretation of the statutes rests on personal judgement and prejudice. When in 1926, the *Klass* rebelled against the committee's long-standing rejection of J. Perrin for a prize, Gullstrand insisted, in an attempt to block him once again, that Perrin's work on brownian motion has not benefitted mankind⁶⁷. Recognizing the inconsistent standards used by the committee, Benedicks responded by calling attention to the fact that the committee seemed to use the statutes arbitrarily to dismiss persons they *a priori* did not want to honour with a prize⁶⁸. Summing up the whole controversy, one member of the committee for chemistry remarked in despair "that to sit on a [nobel] C[ommittee] is like sitting on a quagmire [*gungfly*] — one doesn't have firm footing under one's feet"⁶⁹. Similarly, to discuss and to assess the significance of the Nobel Prizes without taking into account the Swedish context is, as well, "*att sitta på en gungfly*"!

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1. *Nature* 287, 667–668 (1980).

2. Crawford, E. *The Beginnings of the Nobel Prize Institution: The Science Prizes, 1901–1915* (Work in progress).

3. *Protokoll, Nobelkommittén för fysik*, 26 September 1910.

4. *Protokoll, Nobelkommittén för fysik*, 8 January 1901.

5. Letter, Hasselberg, B. to Newcomb, S., 26 October 1900 (Newcomb papers, Library of Congress, Washington, D.C.).

6. *Nobelprotokoll, K. Vetenskapsakademiens 4de Klass*, 29 November 1902.

7. *Protokoll, Nobelkommittén för fysik*, 7 September 1921; *Nobelprotokoll, K. Vetenskapsakademiens 3de Klass*, 29 October 1921.

8. *Protokoll, Nobelkommittén för fysik*, 26 September 1910.

9. *Protokoll, Nobelkommittén för fysik*, 20 September 1911.

10. Arrhenius, S. *Levnadsrön*, 168 (unpublished autobiography, 1927) Arrhenius papers, K. Vetenskapsakademien, Stockholm.

11. Letter, Hasselberg, B. to Hale, G.E., 5 July 1907 G.E. Hale papers.

12. Letter, Hasselberg to Hale, 29 December 1907.

13. *Protokoll, Nobelkommittén för fysik*, 24 September 1908.

14. Letter, Ekholm, N. to Arrhenius, S., 10 March 1910 Arrhenius papers.

15. *Protokoll, Nobelkommittén för fysik*, 8 September 1920.

16. *Protokoll, Nobelkommittén för fysik*, 16 March 1921; 21 September 1911.

17. *Protokoll, Nobelkommittén för fysik*, 7 September 1921.

18. *Protokoll, Nobelkommittén för fysik*, 6 September 1922.

19. Mittag-Leffler, G. *Dagbok: Resansommaren 1922*, 27 July 1922 Kungliga Biblioteket, Stockholm.

20. Letter, Hasselberg to Hildebrandsson, H.H., 9 November 1921 Hildebrandsson papers, Universitetsbiblioteket, Uppsala.

21. *Protokoll, Nobelkommittén för fysik*, 7 September 1921.

22. *Nobelprotokoll, KVA's 3de Klass*, 29 October 1921.

23. Letter, Oseen, C.W. to Arrhenius, 20 November 1922 Arrhenius papers; Oseen, "Den Einsteinska lägen" in *Kosmos. Fysiska uppsatser utgivna år 1922* 105–131 (Stockholm, 1922).

24. Oseen, C.W. *Atomistiska föreläsningar i nutidens fysik. Tid, rum och materia* 15 (Almqvist and Wiksell, Uppsala, 1919).

25. *Protokoll, Nobelkommittén för fysik*, 15 September 1919; 7 September 1921.

26. *Protokoll, Nobelkommittén för fysik*, 6 September 1922.

27. Letters, Oseen to Mittag-Leffler, 15 November 1918, 17 October 1918 Mittag-Leffler papers; Oseen to Bjerknes, V., 12 November 1920, 3 February 1923 Bjerknes papers, Universitetsbiblioteket, Oslo; Oseen to Arrhenius, 29 September 1920 Arrhenius papers.

28. Letter, Oseen to Bjerknes, 3 February 1923.

29. *Protokoll, Nobelkommittén för fysik*, 24 March 1917.

30. *Protokoll, Nobelkommittén för fysik*, 1922, 6 December 1922.

31. *Protokoll, Nobelkommittén för fysik*, 5 September 1923.

32. *Protokoll, Nobelkommittén för fysik*, 31 January 1924.

33. Letter, Oseen to Bjerknes, 18 June 1919 Bjerknes papers.

34. Letter, Granqvist, G. to Arrhenius, 28 December 1921 Arrhenius papers.

35. Letters, Hildebrandsson to Hasselberg, 22 January 1922 Hasselberg papers, Universitetsbiblioteket, Uppsala; Hildebrandsson to KVA's 3de Klass, 4 January 1922 KVA.

36. *Nobelprotokoll, KVA's 3de Klass*, 7 January 1922.

37. Letter, Gullstrand, A. to Arrhenius, 28 March 1923 Arrhenius papers.

38. Letters, Hildebrandsson to KVA, 6 September 1923; Hildebrandsson to KVA's 3de Klass, 6 September 1923 KVA.

39. *Protokoll, KVA's 3de Klass*, 3 September 1923.

40. Letter, Gullstrand to Arrhenius, 25 December 1923 Arrhenius papers.

41. Letters, Oseen to Gullstrand, 1 November 1928, 5 November 1928 Gullstrand papers, Universitetsbiblioteket, Uppsala; *Nobelprotokoll, KVA's 3de och 4de Klasser*, 16 November 1929; *Nobelprotokoll, KVA's 3de Klass*, 30 November 1929.

42. Friedman, R.M., "Vilhelm Bjerknes and the Bergen School of Meteorology, 1918–1923. . . ." (Thesis, Johns Hopkins University, 1978) (book in progress).

43. Letters, Sandström, J.W. to Bjerknes, 22 September 1918 Bjerknes papers; Ryder, C. to Hildebrandsson, 28 July 1922 Hildebrandsson papers.

44. *Protokoll, Nobelkommittén för fysik*, 5 September 1923, 8 September 1924.

45. Letter, Oseen to Ekman, W.V., 3 December 1924 Ekman

papers (private).

46. *Protokoll, Nobelkommittén för fysik*, 5 September 1923.

47. Letter, Pettersson, O. to Ekman, 17 March 1925 Ekman papers.

48. Letter, Oseen to Bjerknes, 16 January 1919 Bjerknes papers.

49. *Nobelprotokoll, KVA's 3de Klass*, 29 October 1925.

50. Letter, Pettersson to Arrhenius, Midsommar 1926 Arrhenius papers.

51. Letter, Pettersson to Ekman, 17 March 1925, 23 November 1930, n.d. Ekman papers.

52. Letter, Pettersson to Ekman, 17 March 1925.

53. Letter, Arrhenius to Bjerknes, 5 November 1925 Bjerknes papers; *Protokoll, KVA's 3de Klass*, 29 October 1925.

54. Letter, Oseen to Ekman, 5 May 1923 Ekman papers.

55. *Dagens Nyheter* (Stockholm), 27 December 1923.

56. *Dagens Nyheter* 28 December 1923.

57. *Protokoll, KVA's 3de Klass*, 20 November 1925.

58. Letter, Ekman to Oseen, 6 April 1936 Oseen papers, KVA.

59. Letters, Oseen to Bjerknes, 4 February 1938 Bjerknes papers; Ekman to Oseen, 7 November 1937, 15 November 1937 Oseen papers.

60. Letters, Ekman to Oseen, 15 November 1937, 25 January 1938 Oseen papers.

61. Letter, Sandström to Bjerknes, 4 April 1939 Bjerknes papers; *Dagens Nyheter* 3 March 1939.

62. Oseen, "Albert Viktor Bäcklund" in *Vetenskapsakademiens Arsbok 1924* 303–304, 306–307 (Uppsala, 1924).

63. Letter, Oseen to Bohr, Niels, 25 June 1935. Bohr Scientific Correspondence Niels Bohr Institute, Copenhagen.

64. Letter, Ekman to Oseen, 18 October 1939 (quoting from "Kompletterande utredning om V. Bjerknes") Oseen papers.

65. Letters, Bjerknes to Oseen, 17 January 1938 Oseen papers; Oseen to Bjerknes, 4 February 1938 Bjerknes papers.

66. Letter, Oseen to Bjerknes, 4 February 1938.

67. Letter, Oseen to Arrhenius, 3 November 1926 Arrhenius papers; *Nobelprotokoll, KVA's 3de Klass*, 23 October 1926.

68. *Nobelprotokoll, KVA's 3de Klass*, 23 October 1926.

69. Letter, Widman, O. to Palmer, W., 19 November 1826 [sic] Palmer papers KVA.

NEWS AND VIEWS

The search for proton decay

from Frank Close

THE discovery that at ultra-high energy, the strong, electromagnetic and weak forces may take on similar behaviours has led to the development of grand unified theories (GUTs) which unite them at 10^{15} GeV. The theories predict that, at extreme energies, quarks and leptons assume similar characteristics and can transmute back and forth. At very low energies, such as are present today, these exotic processes are frozen but not totally eradicated. Transmutation of quarks (in the proton) into leptons is predicted to occur, albeit very rarely, causing protons to decay with a half life of about 10^{30} yr.

The search for evidence of proton decay is one of the most popular topics in high-energy physics and the opening talk at the biennial European Physical Society conference* held in Lisbon last month outlined ten independent such experiments around the world. Three are still proposals, five are being set up, one has put a limit of $\geq 10^{30}$ yr on the lifetime and a Bombay-Japan collaboration has three possible candidate events for proton decays. If these survive, then the future will be very exciting as the proposed experiments should see many scores of events which will not only confirm the proton decay phenomenon but, by studying the nature of its decay products, yield profound insights into the relationship between quarks and leptons.

Studying rare phenomena, such as the cold residue of a high-energy unity, is one way of testing the unified theories. More direct of course is to push laboratory experiments to ever higher-energy regimes and so push nearer to that unity.

H. Schopper, the director-general of CERN, surveyed the planning for high-energy physics facilities until the 1990s. Towards the end of this decade it is hoped that very high-energy annihilations of electrons and positrons may be achieved at Stanford University, California or at the large electron-positron accelerator (LEP)

to be built at CERN, Geneva, which will be able to probe the energy region where the weak force has merged in strength with the electromagnetic. Great excitement was generated by Carlo Rubbia's (CERN) announcement that the first collisions of protons and antiprotons had been achieved in the CERN SPS accelerator, paving the way for producing W and Z bosons there. The optimists hope that the first few events producing these weak interaction quanta may occur by early next year.

The theories of electromagnetic and nuclear forces that have spawned the GUT were reviewed by several speakers. On the phenomenological front, P. Landshoff (University of Cambridge) surveyed the field, concluding that all the data continue to support the Glashow-Weinberg-Salam $SU_2 \times U_1$ model of electroweak forces and the SU_3 quantum chromodynamic theory (QCD) of quark forces. The obvious qualitative successes of the latter are proving hard to make more quantitative because perturbation theory of quark-gluon interactions converges very slowly. Landshoff reviewed some theoretical attempts to construct approaches to the renormalization of QCD which would improve the convergence properties. This is an area of study that is still in some flux.

R. Marshall (Rutherford Laboratory, UK) exhibited some of the most compelling evidence yet for gluons manifesting themselves as parents of particle jets in electron-positron annihilation data. The electron and positron annihilate producing a quark in one direction and an antiquark and glue centred on the opposite direction. A shower of particles induced by the quark is balanced by two showers symmetric about the 180° axis — the products of (anti) quark and gluon.

F. Close (Rutherford Laboratory) pointed out how just as coloured quarks cluster to form 'white' hadrons, so should coloured gluons cluster to give white glueballs. There has been some speculation that a glueball may have been seen with a mass of about 1,400 MeV (*Nature, News and Views* 292; 195, 1981). It has also been

suggested that a 2-GeV state seen decaying into two ϕ mesons may be a glueball; more data from this experiment may elucidate the possibility. At the moment the 1,400-MeV state looks the best bet but, as outlined in *Nature* (292; 195, 1981), there is still much work to be done.

The search for white glueballs as opposed to individual coloured gluons is based on the belief that QCD confines colour in such clusters. G. 't Hooft (University of Utrecht) gave a detailed review of current attempts to prove this and R. Feynman (Caltech) reported his own attempts to prove confinement in a world of two-spatial dimensions.

But are quarks confined? A confrontation took place between W. Fairbank (Stanford University), who has claimed to observe fractional electrical charges on niobium spheres, and G. Morpurgo (University of Genoa), who finds no such phenomenon on iron. The issue was not resolved — Morpurgo's experiment cannot levitate niobium, and Fairbank's cannot levitate iron. Morpurgo may be able to study an 80:20 iron/niobium mixture soon; whether this helps resolve the question remains to be seen. (See Lyons *Nature, News and Views* 291; 534, 1981 for a discussion.)

Another interesting confrontation between groups with data in *prima facie* conflict took place. A Bologna-CERN-Frascati collaboration has claimed to produce a baryon containing a bottom (or 'beauty' quark). This state has a mass of 5.4 GeV and, if verified, would be the most massive baryon ever found. The experiment occurred in the split-field magnet at the CERN intersecting storage rings (ISR) and the claimed mass is in excellent agreement with theoretical predictions. However, a collaboration from Annecy-CERN-Paris-Dortmund-Heidelberg and Warsaw reported that they had performed a similar experiment in the same conditions and found no signal.

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*The High-Energy Physics European Physical Society conference was held in Lisbon from 8 to 15 July.

Transposable elements and proviruses

from D.J. Finnegan

THE discovery that both prokaryotic and eukaryotic genomes are less stable than classical genetic analysis had indicated is one of the many important developments made in molecular genetics over the past few years. Application of recombinant DNA techniques to the study of eukaryotic genome organization has revealed the presence of transposable elements within the genome of *Drosophila melanogaster*¹⁻³. These comprise about thirty families of repeated sequences, together making up approximately one-half of the moderately repetitive DNA (5–10 per cent of the total genome) in this species. The best studied families (known as *copia*, 412, 297, *mdg1* and *mdg3* after the first member of each to be studied) have several properties in common, although there is no detectable homology between them. The members of each family (often referred to generically as 'copia-like' elements) are well conserved and are located at 20–40 sites distributed throughout the genome. The number and locations of elements in each family vary between embryonic and tissue culture cell DNA where there may be up to 250 copies per haploid genome^{2,4}. The number of potential sites must be high and elements may even insert at random.

At both ends of a transposable element are direct repeats (1 in Fig. 1a) a few hundred base pairs long, the length and sequence of which are specific for each family of elements^{5,6}. At the extreme ends of each element are short (about 10 base pairs) inverted repeats (2 in Fig. 1a) which, except in the *mdg3* family, occur at both ends of the long direct repeats. Immediately before and after each element is a short direct repeat (3 in Fig. 1a), the length, but not sequence, of which is constant for all members of a particular family (5 base pairs in the case of *copia* elements). Comparisons between particular sites in the genome from different sources, one containing and one lacking (an 'empty site') a transposable element, indicate that the bases of the short direct repeat occur only once at the site into which an element inserts⁷.

The properties of *Drosophila* transposable elements described above resemble those of bacterial transposons⁸. This raises the possibility that, despite no evidence for a direct relationship between the two, copia-like elements might be responsible for the same range of genetic events (stable and unstable mutations, deletions, inversions and translocations) in *D. melanogaster* as are transposons in *Escherichia coli*. The most interesting

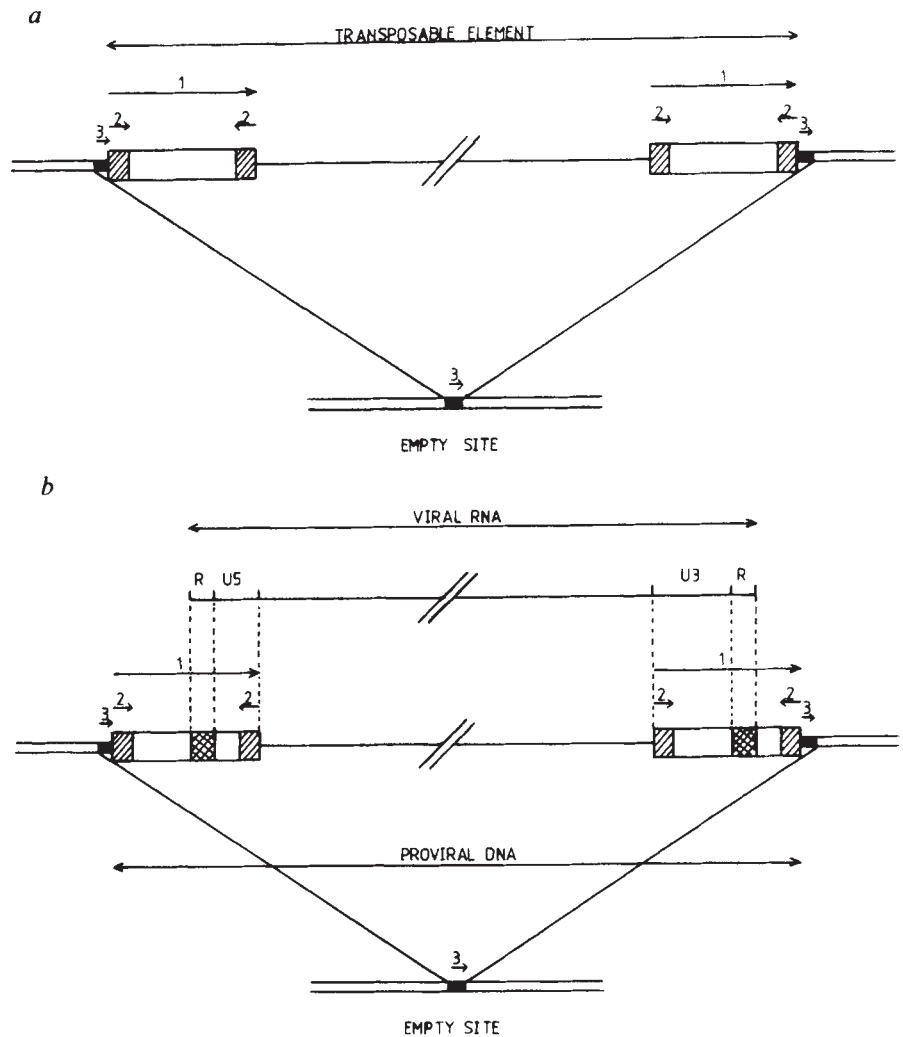


Fig. 1 a, A transposable element. The various components are: 1, long direct repeat; 2, inverted repeat; 3, short direct repeat. b, Relationship between viral RNA and proviral DNA. U3, unique sequence from the 3' end of the viral RNA; R, sequence repeated at both ends of the viral RNA; U5, unique sequence from the 5' end of the viral RNA.

example of this to date concerns the *white eye* gene (*w*). Bingham, Judd and Rubin (Rubin, personal communication) have shown that the *white apricot* mutation, *w^a*, is due to insertion of a *copia* element at the *white* locus. Some unstable *w* alleles are also due to insertions, although the sequences involved do not belong to any of the well characterized families of transposable elements (Rubin, personal communication).

The abundance of transposable elements in the genome of *D. melanogaster*, and the fact that their activity can have recognizable genetic consequences, prompt one to ask whether sequences of this type might be found in other eukaryotes. Indeed, a family of transposable elements, *Ty1* elements, has been discovered in the yeast *Saccharomyces cerevisiae* and these have properties very similar to those of copia-like elements. The same is true of the DNA

proviruses of vertebrate retroviruses.

Cells which have been infected by retroviruses may have one or more copies (proviruses) of the viral genome integrated into their chromosomes. At each end of a provirus are direct repeats, known as long terminal repeats (LTRs). At the left-hand end of a LTR is a unique sequence from the 3' end of the viral RNA. Adjacent to this is the sequence repeated at both ends of the RNA and a unique sequence from its 5' end. These are shown in Fig. 1b where they are designated U3, R and U5 respectively. After infection of a host cell, viral RNA molecules are first copied into linear double-stranded DNAs with a LTR at each end. These are then converted into circular molecules with either one copy of the LTR or two copies in tandem. It is not known which of these DNAs integrates into host chromosomes but some evidence favours a circular form (for detailed

discussion, see ref. 9). The sequence organization of proviruses resembles that of *Drosophila* transposable elements in several ways¹⁰. They have short inverted repeats at their ends and are flanked by a direct repeat of a few base pairs which occurs only once at the site of insertion in their absence (Fig. 1b).

Vertebrates do not have to be infected by retroviruses to acquire sequences of this type. The genomes of most, if not all, vertebrates contain endogenous proviruses, which can make up a substantial proportion of their DNA (about 0.1 per cent in primates and rodents)¹¹. Like *Drosophila* transposable elements, the chromosomal locations of endogenous proviruses differ between individuals in populations of mice and chickens. The topographical similarities between proviruses and transposable elements suggest that they may be related and Temin¹² has argued that proviruses evolve from transposable elements. If this is the case, one might expect to find within *D. melanogaster* cells circular DNAs similar to extrachromosomal proviral DNA. Circular DNAs were detected in *D. melanogaster* embryos and tissue culture cells some time ago and have been shown to be largely comprised of repetitive sequences¹³. In a recent issue of *Nature* (292; 591, 1981), Flavell and Ish Horowicz described their attempts to find molecules with *copia*-specific sequences amongst these circles. They have been able to purify three different circular DNAs of this type from *D. melanogaster* tissue culture cells using ethidium bromide-caesium chloride density centrifugation and DNA cloning techniques. On the basis of their restriction site maps two of them do appear analogous to circular proviral DNA, having all the sequences from the body of a *copia* element plus one copy of its long direct repeat or two repeats in tandem. The third class appears to be a deletion derivative of one of the other two.

Stimulating though these observations are, they do not clarify the relationship between transposable elements and retroviruses since (as Flavell and Ish Horowicz point out) these circular molecules need not be produced by reverse transcription but could be formed by

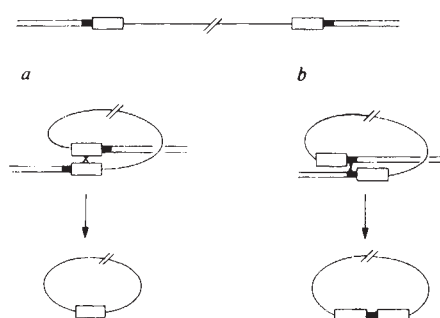


Fig. 2 Recombination in a transposable element: *a*, between the long direct repeats at the ends of an element, giving a circular molecule with one copy of the direct repeat, and *b*, between the 5 base pairs on either side of the element giving a circular molecule with two copies of the direct repeat.

recombination. Recombination between the long direct repeats at the ends of a transposable element would yield a circular molecule with one copy of the direct repeat (Fig. 2a) whereas recombination between the 5 base pairs repeated on either side of a *copia* element would yield a circular molecule with two copies of the direct repeat in tandem (Fig. 2b). These possibilities should be distinguished by DNA sequence analysis of the circular molecules with two copies of the long direct

repeat. If they are derived by recombination, then the two repeats should always be separated by 5 base pairs but a different 5 base pairs in each case; if they are derived by reverse transcription of full-length *copia* RNA, then all circles with two repeats should be identical. Whatever their origin these circles could be intermediates in transposition, a possibility which Flavell and Ish Horowicz are investigating.

The sequences discussed so far are not the only ones involved in DNA rearrangements. Potter and his colleagues have found transposable sequences in *D. melanogaster* with properties distinctly different from those of *copia*-like elements and specific, rather than random or semi-random, rearrangements have been demonstrated for genes controlling mating type in *S. cerevisiae*, surface antigens in trypanosomes and immunoglobulins in mammals. Others may exist, but one should be wary of getting too carried away by these results. Eukaryotic genomes are more labile than has been generally supposed but wholesale rearrangements do not occur. In *Drosophila*, genes still retain their order in linkage groups, this order is the same in somatic and germ-line cells, and the banding pattern of polytene chromosomes is remarkably constant. □

The expanding use of potential-sensitive dyes

from a correspondent

THE recent paper¹ in *Nature* reporting the use of a voltage-sensitive dye to localize pacemaker activity in early embryonic heart is a good example of the expanding interest in and application of potential-sensitive dyes for biological, electrophysiological and biophysical studies. Cyanine, merocyanine and oxonol dyes respond spectrally to potential changes in, for example, squid giant axon^{2,3}, red blood cells⁴⁻⁶, black lipid membranes⁷, frog skeletal muscle⁸ and frog heart⁹.

The available probes are of two general types: those which permeate the cell membrane and have both slow and fast responses (for example, carbocyanines) and non-penetrating dyes with only fast signals, of which merocyanine-rhodanine used by Kamino *et al.*¹ is a good example. As reviewed recently by Waggoner¹⁰, the slow dyes, with their large responses, seem ideally suited for following the kinetics of potential changes that occur in times of a few seconds, whereas the fast probes can resolve adequately events associated with membrane excitation during action

potentials, although their absorption and fluorescence changes are usually only 0.001 to 1 per cent. The slow (or redistribution or accumulation) dyes operate by potential-dependent mechanisms redistributing the charged dye between extracellular and intracellular environments of the cell, organelle or vesicle, whereas optical signals from rapidly responding dyes occur through potential-dependent changes of dye molecules located on (or very near) the membrane.

There are obvious attractions in using these optical probes, especially in cells which are too small to be examined by classical microelectrode techniques, or in complex cellular networks where multiple and accurate impalements would be very laborious and difficult. However, it has long been recognized that care has to be taken in both interpreting experimental data, hence the comforting corroboration in squid axon where parallel membrane potential measurements can be made with confidence, and ensuring that introduction of the dye does not in itself perturb the preparation. Permeant dyes can inhibit

1. Potter, S.S., Brorien, W.J., Dunsmuir, P. & Rubin, G.M. *Cell* 17, 415 (1979).
2. Tchrikov, N.A., Ilyin, Y.V., Ananiev, E.V. & Georgiev, G.P. *Nucleic Acids Res.* 6, 2169 (1978).
3. Rubin, G.M. *et al.* *Cold Spring Harb. Symp. quant. Biol.* 45 (in the press).
4. Strobel, E., Dunsmuir, P. & Rubin, G.M. *Cell* 17, 429 (1979).
5. Levis, R., Dunsmuir, P. & Rubin, G.M. *Cell* 21, 581 (1980).
6. Bayev, A.A. *et al.* *Nucleic Acids Res.* 8, 3263 (1980).
7. Dunsmuir, P., Brorien, W.J., Simon, M.A. & Rubin, G.M. *Cell* 21, 576 (1980).
8. Calos, M.P. & Miller, J.H. *Cell* 20, 579 (1980).
9. Gilboa, E., Mitra, S.W., Goff, S. & Baltimore, D. *Cell* 18, 93 (1979).
10. Shimotohno, K., Mizutani, S. & Temin, H.M. *Nature* 285, 550 (1980).
11. Todaro, G.J., Callahan, R., Rapp, U.R. & De I. Arco, J.E. *Proc. R. Soc. B210*, 367 (1980).
12. Temin, H.M. *Cell* 21, 599 (1980).
13. Stanfield, S.W. & Lengyel, J.A. *Proc. natn. Acad. Sci. U.S.A.* 76, 6142 (1979).

intracellular metabolic processes and the binding of charged dye molecules might interfere with important membrane processes mediating biological responses. In addition, dyes may react with the exogenous reagents, whose effects are being examined, to form non-fluorescent complexes. This would make dose-response curves difficult to interpret and would complicate kinetic calculations. Furthermore, even with modest illuminations, some dyes can cause cellular photodynamic damage. These considerations have prompted intensive work to produce dyes with better responses and biological inertness.

It is possible to end on an optimistic note, for recent reports (to quote but two examples) have described how potential-sensitive dyes can be used to advantage to monitor either mitochondrial membrane

potentials¹¹ or radial propagation along muscle fibre T-tubules¹². These, together with the elegant studies of Kamino, Hirota and Fujii¹, suggest that such techniques have an important future in many investigations involving molecular mechanisms at the cellular level. □

1. Kamino, K., Hirota, A. & Fujii, S. *Nature* **290**, 595 (1981).
2. Cohen, L.B. *et al. J. Membrane Biol.* **19**, 1 (1974).
3. Ross, W.N. *et al. J. Membrane Biol.* **33**, 141 (1977).
4. Hoffman, J.F. & Laris, P.C. *J. Physiol., Lond.* **239**, 519 (1974).
5. Sims, P.J. *et al. Biochemistry* **13**, 3315 (1974).
6. Hladky, S.B. & Rink, T.J. *J. Physiol., Lond.* **263**, 287 (1976).
7. Waggoner, A.S. *et al. J. Membrane Biol.* **33**, 21 (1977).
8. Baylor, S.M. & Chandler, W.K. *Biophys. J.* **25**(2), 119a.
9. Morad, M. & Selema, G. *J. Physiol., Lond.* **292**, 267 (1972).
10. Waggoner, A.S. *et al. Rev. Biophys. Bioengng* **8**, 47 (1979).
11. Johnson, L.V. *et al. J. Cell Biol.* **88**, 526 (1981).
12. Nakajima, S. & Gilai, A. *J. gen. Physiol.* **76**, 751 (1980).

The evolution of sedimentary basins

from C.A. Williams

THE selection of 'The evolution of sedimentary basins' as the topic for a Royal Society discussion meeting* reflects the impetus given to the field by new models of basin formation, in particular the crustal stretching model of McKenzie, and by the availability of quantitative data on crustal loading and flexure which are allowing models to be tested. The McKenzie model (*Earth planet. Sci. Lett.* **40**; 25, 1978) proposes that sedimentary basins are formed by rapid stretching of the continental lithosphere by normal faulting, instigated by a passive upwelling of hot asthenospheric material beneath the crust. Once the thermal stage has passed, the lithosphere thickens as the heat is conducted to the surface and undergoes a slow subsidence which is not associated with faulting and which may be amplified by sediment loading.

The meeting made no attempt to cover all types of basin, but concentrated on those where good-quality data are available. Discussion of continental margin basins was dominated by contributions on the Biscay margin (presented by D.G. Roberts, IOS, and J.-P. Foucher, CNEXO, Brest), largely based on the results of IPOD drilling on the North-west Biscay margin and on associated seismic reflection and refraction data. Both Roberts and Foucher showed seismic reflection evidence of listric faults dipping consistently oceanwards. These two

studies, whilst producing the most detailed information we yet have on this margin, also indicate the limitations of fragmentary evidence in reassembling faulted strata. Robert's interpretation requires infinite stretching of the lower crust but less stretching in the upper crust than that required by Foucher's interpretation, yet both studies are based on essentially the same data set. The range of disagreement is such as to encourage the acquisition of more and better data to allow more accurate estimation of fault displacements.

The continental margin basin section was completed by an exposition of the North-west Australian margin by D.E. Powell (Hudbay), a margin remarkable in that the basins seem to be related to the late Palaeozoic phase of abortive rifting with little or no modification during the eventually successful rifting in the early Cretaceous.

An intriguing puzzle came from discussion of intracontinental basins. There appear to be two types of basement beneath basins, one in which normal faulting occurs as expected; but in cratonic shelf basins, for example, Witwatersrand, Transvaal and Michigan Basins, the basement does not appear to be normally faulted and some other mechanism of basement subsidence, other than extension by faulting, must be evoked. The Michigan Basin, nonetheless, shows the same exponential subsidence as other basins and the existence of COCORP seismic reflection data across it suggests a clear line of future study into the apparent lack of basement faulting.

In the session on basin formation, A.B. Watts (Lamont-Doherty Geological Observatory) discussed the role of crustal flexure, showing that this becomes a major factor in basins more than 200 km wide and that the best overall fit to the observations is an elastic plate model with crustal strength increasing with age. This predicts a sedimentary geometry with coastal onlap and it seems that Vail's eustatic sea-level changes based on evidence of onlap may in reality be a record of basin subsidence. The thermal effects of subsidence were discussed by D.L. Turcotte (Cornell University) (all basins appear to be initiated by a thermal event), while C. Beaumont (Dalhousie University) compared foreland and Atlantic-type basins and concluded that the major contrast is the thermal age of the lithosphere. The old, cooler and thicker lithosphere of the foreland compressive area will respond to loading with greater rigidity and depression wavelength than the younger, hotter, thinner and less rigid rifted continental margin basin.

Some of the outstanding problems in understanding sedimentary basin subsidence, in particular the origin of tensile stresses in the crust which initiate the normal faulting, were discussed by M.H.P. Bott (Durham University). Their origin may be found in stresses at convergent plate boundaries, hence, for example, the origin of the Northern European Carboniferous basins may be related to stress fields in operation during the subduction of the Hercynian Ocean.

In summarizing the meeting, A.W. Bally (Shell) cautioned that the folded belts, for example Zagros, Franklinian Basin and the Precambrian basins, are too complicated for modelling with currently available data. The complexity of listric faulting causes inherent problems in estimating fault displacement. While in some spectacular cases the sole of the listric fault has been observed, more high-quality seismic data across basins are required for better evidence of the actual existence and shape of listric faults. The question of whether symmetric graben are purely a function of geological imagination was also raised, since observed graben are either asymmetric or exist as half graben.

The general conclusion was that the stretching model gives a reasonable explanation of the formation of many basins. It was a little disappointing that among the excellent and often complicated stratigraphical histories described in oil-company presentations, studies on subsidence, flexure, fault displacements and so on were not emphasized, leaving one to wonder whether the oil companies are way ahead in considering these effects in a quantitative way, though refrained from divulging same, or whether in fact it is the academics who are leading in this area. □

*The meeting was organized by Sir Peter Kent, M.H.P. Bott, D.P. McKenzie and C.A. Williams and the proceedings will be published in the *Philosophical Transactions of the Royal Society*, January 1982.

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Membrane manoeuvres in Marseille

from R.C. Hider

MOST animal and bacterial toxins are directed against membranes, the more toxic interfering with critical receptor proteins while others are highly efficient lytic agents. The former, by virtue of their high-affinity interactions, have been used as probes in developmental and physiological studies and the latter, because of their affinity for lipids, are used in the elucidation of the molecular organization of membranes. A number of recent developments in these fields were reported at a meeting of the International Society of Toxinology* where discussions centred on the sodium channel, the cholinergic synapse and cytolytic toxins formed the framework of the meeting.

The sodium channel

The voltage-dependent sodium channel, a prime constituent of excitable membranes, remained firmly out of the biochemist's reach until the discovery of channel-specific toxins. Since 1970 however, the concerted application of tetrodotoxin, saxitoxin, scorpion and sea anemone toxins has given reliable information on the distribution, density and unit ion flux of

various sodium channels. As reported by V. Berwald-Netter (Collège de France, Paris) and J.F. Renaud (Centre de Biochimie, Nice), the development of these channels in embryonic tissue can also be monitored as sea anemone and scorpion toxins bind to both silent and functional channels¹ and can give a quantitative index of neuronal maturation². W.A. Catterall (University of Washington, Seattle) reported the isolation of a functional sodium channel from mammalian brain³ but instability of the preparation — its lifetime is limited to 3 days — is a major problem. As with other membrane protein isolation procedures, the presence of a protease inhibitor cocktail has been essential, Catterall's laboratory including *o*-phenanthroline. This powerful chelator removes essential cationic cofactors from proteolytic enzymes and yet leaves calcium in solution to enhance the stability of the isolated channel. Catterall reported that the membrane channel consists of two proteins of molecular weights 270,000 and 37,000, both being specifically labelled by photoaffinity-tagged sea anemone toxin. The nature of the large subunit, which has also been reported elsewhere⁴, is of great interest. Does it consist of subunits cross-linked by functional groups other than disulphide fuctions? Does calcium

modulate its conformation? Whatever the answers to these questions, its isolation in a functional state marks the beginning of a new era which is likely to be centred on precise electrophysiological experiments with well defined reconstituted bilayer membranes.

There was general agreement at the meeting that the 'old world' scorpion neurotoxins bind to the sodium channel in a voltage-dependent manner and thus interact with a component capable of existing in two conformational states, the relative proportions of which depend on membrane potential⁵. However, the 'new world' scorpion toxins lack this property and bind to a different site on the sodium channel⁶. The three-dimensional structure of one such toxin (from *Centruroides sculpturatus*) was shown by J.C. Fontecilla (Aarhus University) to possess an extensive backbone of secondary structure cross-linked by four disulphide bridges⁷. Emerging from this relatively rigid structure are two peptide loops which are believed to interact with the receptor. On the basis of secondary structure predictions, Fontecilla suggested that both

*The Fourth European Symposium on Animal, Plant and Microbial Toxins (24–27 June 1981) was organized by Hervé Rochat, Faculté de Médecine Secteur Nord, Marseille. The proceedings of the meeting will be published by Pergamon Press in 1982.

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100 years ago

"How I crossed Africa, from the Atlantic to the Indian Ocean"

By Major Serpa Pinto
(London: Sampson Low & Co., 1881)

To cross Africa has almost ceased to be an extraordinary feat. Indeed it seems evident, the more we know of the Portuguese native traders, that even before Livingstone's memorable first journey, it was no uncommon thing for the Pombeiros to do in the ordinary way of business. Of course some routes are more dangerous than others, and that by which Stanley made his famous march was perhaps the most difficult and dangerous that could be selected. Still the journey performed by Major Serpa Pinto was in many ways remarkable, and perhaps not its least remarkable feature is the characteristic manner in which he tells his story.

One of our illustrations gives a good idea of an antelope which was met with in the Cuchibi, which the Major thus describes:

"At one of the turns of the river I perceived three antelopes of an unknown species, at least to me; but just as I was in the act of letting fly at them they leaped into the water and disappeared beneath its surface. The circumstance caused me immense surprise, which was increased as I went further on, as I occasionally came across several of these creatures, swimming; and then rapidly diving, keeping their heads under water, so that only the tips of their horns were visible. This strange animal bears among the Bihenos the name of Quichôbo. Its life is in a great measure passed in the water, it never straying far



The Quichôbo

from the river banks, on to which it crawls for pasture, and then chiefly in the night-time. It sleeps and reposes in the water. Its diving-powers are equal, if not superior, to those of the hippopotamus. During sleep it comes near to the surface of the water, so as to show half its horns above it. It is very timid by nature, and plunges to the bottom of the river at the slightest symptom of danger. It can easily be captured and killed, so that the natives hunt it successfully, turning to account its magnificent skin and feeding off its carcase, which is however but poor meat. Upon leaving the water for pasture its little skill in running allows the natives to take it alive; and it is not

dangerous, even at bay, like most the antelope tribe. The female, as well as the male, is furnished with horns. There are many points of contact between the life of this strange ruminant and that of the hippopotamus, its near neighbour. The rivers Cubangui, Cuchibi, and the upper Cuando offer a refuge to thousands of Quichôbos, whilst they do not appear either in the lower Cuando or the Zambesi. I explain this fact by the greater ferocity of the crocodiles in the Zambesi and lower Cuando, which would make short work of so defenceless an animal if it ventured to show itself in their waters".
From *Nature* 24, 7 July, 215, 1881.

classes of toxin have similar shapes but differ in the relative lengths of the peptide loops emerging from the conserved framework. Why these relatively minor differences are associated with the presence or absence of voltage-sensitive binding is not clear but it would be interesting to study their relative affinity for the 37,000-molecular weight protein identified by Catterall.

The cholinergic synapse

There is now general agreement on the structure of the *Torpedo* (electric ray) acetylcholine receptor. In his review, J.P. Changeux (Pasteur Institute) reported that the basic functional unit, as judged by reconstitution experiments, is composed of four polypeptide subunits of molecular weights 40, 50, 60 and 65×10^3 respectively, the latter three being extremely susceptible towards proteolysis. The 40,000-molecular weight protein is the site of agonist (acetylcholine) and antagonist (curare and elapidae snake toxin) binding. Exactly how the snake neurotoxins interact with the 40,000-molecular weight subunit is unknown and this uncertainty severely limits their use as affinity ligands. However the problem is being energetically attacked by the Russian group led by F. Bystrov and V. Tsetlin (Shemyakin Institute), who are applying ESR, NMR and photoaffinity labelling techniques to monitor the toxin-receptor interaction⁸. A 43,000-molecular weight subunit, which is also associated with the receptor, has been shown not to be essential for acetylcholine receptor-mediated cation translocation⁹ but is thought to have a structural role, cross-linking receptor rosettes into the characteristic double rows often seen in electron micrographs¹⁰.

Presynaptic phenomena are not so well characterized at the molecular level as are the corresponding postsynaptic events, but J.O. Dolly (Imperial College, London) and A.L. Harvey (University of Strathclyde) reported two new approaches designed to tackle this problem. Dolly described a method for the purification and iodination of botulinum toxin which specifically inhibits the release of acetylcholine¹¹ and Harvey demonstrated that Kunitz-type protease inhibitor homologues, isolated from the venom of *Dendroaspis* snakes (mambas), dramatically stimulate acetylcholine release¹². Significantly, as judged from pharmacological studies, these inhibitor-like polypeptides compete with β -bungarotoxin (from krait venom) for a common binding site on the presynaptic membrane. β -bungarotoxin is used extensively by physiologists and consists of two polypeptides, the A chain, a phospholipase, and the B chain, a protease inhibitor homologue. Thus it seems likely that the B chain directs the phospholipase activity of the A chain to the presynaptic membrane. Both botulinum toxin and protease inhibitor homologues would appear to have potential as specific presynaptic probes, as unlike the toxins currently used,

they lack phospholipase activity.

Development of new antivenoms

In many meetings devoted to neurotoxins, there is often a tendency to forget that snakes and scorpions can present serious health hazards. Each year 1,000 people die in Mexico from scorpion stings and 2,500 people die in South-east Asia from snake bites¹³. Some of the symptoms associated with such poisoning were vividly presented in a session devoted to the clinical aspects of envenomation which was chaired by H.A. Reid (Liverpool School of Tropical Medicine and see ref. 14). The efficiency of antivenoms is likely to improve now that new techniques have been developed to produce antibodies specifically directed against the most toxic components of the venom. One such example was reported by J.C. Boulain and A. Menez (CEN Saclay, France) where the production of a monoclonal antibody directed against a cobra neurotoxin was described. Combinations of such antibodies directed against different snake neurotoxins should produce efficient antivenoms for specific geographical localities and thus, in principle, a comprehensive range of such antivenoms could become available for world-wide distribution.

Cytolytic toxins

Cobra venoms, in addition to containing postsynaptically acting neurotoxins, possess an even higher content of so-called 'cardiotoxins'. This term, although widely used, provides too narrow a label as these polypeptides depolarize all muscle types and many also possess lytic properties. Indeed, as described by D.S. Chapman (Ashington, Northumberland, UK), cytotoxicity and not neurotoxicity is the dominant symptom of *Naja nigricollis* (spitting cobra) envenomation. J. Dufourcq (Talence, France) eloquently described the interaction of these hydrophobic proteins with negatively charged lipids, showing how they induce lipid phase separation, and R. Hider (University of Essex) demonstrated that some toxins which possess powerful cardiotoxic properties lack lytic properties, arguing against the view that the muscle-depolarizing ability is directly associated with non-

specific lytic properties. In the past much of this work has been bedevilled by contaminating traces of phospholipase, which can endow the preparation with lytic activity. H. Veheij (University of Utrecht) reviewed the chemistry of phospholipase-A₂ molecules, indicating how the increased content of surface aromatic side chains is associated with improved powers of membrane penetration. Presumably the tight association between these enzymes and the hydrophobic cardiotoxins results from a similar interaction. Immunoaffinity chromatography is an excellent method for removing traces of phospholipase¹⁵ and probably should be used routinely in the isolation of this class of protein.

R. Mollby and M. Thelestam (National Bacteriology Laboratory and Karolinska Institute, Stockholm) described how their fibroblast-based analytical system permitted the classification of cytolytic toxins¹⁶. Their data formed a useful background for the classification of toxins isolated from *Aeromonas hydrophila* (Gram-negative rod bacterium) and the so-called thiol-activated cytotoxins. However it was clear from the meeting that the mode of action of many of these toxins is not understood. The colicins A, E, I and K were reported by F. Pattus and C. Lazdunski (University of Marseille) to form voltage-dependent channels in bilayer membranes, in a similar manner to the lytic peptide alamethicin¹⁷. In this context, J.H. Freer (University of Strathclyde) tentatively suggested the existence of an oligomeric pore structure for staphylococcal δ -lysin and R. Hider (University of Essex) made a similar suggestion for bee venom melittin. The lipophilic portion of most plasma membranes is 3 nm thick and thus can be spanned by a 21-residue α -helix. As alamethicin (20 residues), δ -lysin (26 residues) and melittin (26 residues) can all form amphiphilic helices, toxin aggregates could readily form hydrophilic pores capable of spanning a membrane. Significantly, the average number of residues in each of the seven bacteriorhodopsin α -helices is 25 (see ref. 18). Thus in principle, cytotoxin oligomers could form bacteriorhodopsin-like structures. If calcium entered or magnesium left the cell via such channels, intracellular proteases would be activated, inducing dramatic effects on the cell membrane¹⁹. Ion specificity of such a channel would be controlled by both the diameter of the aqueous channel and its net charge, and as such could be related to the Mollby-Thelestam classification scheme. Ion specificity of such oligomeric structures will be of considerable interest.

The success of this meeting was, to a large extent, due to the International Society of Toxinology attracting, and thus facilitating, interaction between scientists from an extremely wide range of disciplines. As toxin chemistry and biochemistry continue to expand, so will this Society.

1. Romey et al. *Biochim. biophys. Acta* 556, 344 (1979).
2. Berwald-Netter et al. *Proc. natn. Acad. Sci. U.S.A.* 78, 1245 (1981).
3. Catterall et al. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
4. Agew & Raftery *Biochemistry* 18, 1912 (1979).
5. Catterall A. *Rev. Pharmac. Toxicol.* 20, 15 (1980).
6. Jover et al. *Biochem. biophys. Res. Commun.* 95, 1607 (1980).
7. Bugg et al. *Proc. natn. Acad. Sci. U.S.A.* 77, 6496 (1980).
8. Bystrov et al. *FEBS Lett.* 106, 47 (1979).
9. Wu & Raftery *Biochemistry* 20, 694 (1981).
10. Changeux et al. *FEBS Lett.* 121, 327 (1980).
11. Dolly et al. *J. Physiol., Lond.* (in the press).
12. Harvey et al. *Naunyn-Schmiedeberg Arch. Pharmac.* 312, 1 (1980).
13. *Progress in Characterization of Venoms and Standardization of Anti-venoms* (W.H.O., 1981).
14. *Toxicon* 18, 129 (1980).
15. Delori & Tessier *Biochimie* 62, 287 (1980).
16. Mollby & Thelestam *Biochim. biophys. Acta* 557, 156 (1979).
17. Latorre & Alvarez *Physiol. Rev.* 61, 77 (1981).
18. Engelman et al. *Proc. natn. Acad. Sci. U.S.A.* 77, 2023 (1981).
19. Duncan *Experientia* 34, 1531 (1978); Schanne et al. *Science* 206, 700 (1979).

ARTICLES

Three myosin heavy-chain isozymes appear sequentially in rat muscle development

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Three different heavy-chain isozymes of myosin appear sequentially in rat muscle during the period from late gestation to about three weeks of age. In part these changes can be related to changes in innervation taking place during this period.

THE myosins found in both muscle and non-muscle cells comprise a family of proteins having similar native structures and subunit compositions¹⁻⁴. The myosin isoenzymes in adult skeletal muscle can be classified into two major types. A myosin capable of rapid ATP hydrolysis is characteristic of fast contracting muscle fibres while a myosin of lower ATPase activity is found in slow-contracting fibres⁵. These fast and slow isoenzymes differ in both their heavy- and light-chain subunits. It has been shown⁵ that the maximum speed of shortening of adult muscles is closely correlated to the rate of hydrolysis of ATP by myosin. Therefore the type of isoenzyme present in a muscle can be of physiological importance^{5,6}.

The muscles of newborn animals contract slowly although they may become fast-contracting in the adult⁷⁻⁹. Previous results¹⁰⁻¹⁶ have suggested that slow myosin heavy chain was a major constituent of developing muscle, whereas others report the presence of fast myosin¹⁷⁻²¹ (see also discussion in refs 22, 23).

Recent biochemical and immunological studies have however shown that fetal and newborn skeletal muscles contain certain myosin subunits distinct from the adult forms²⁴⁻²⁷. In the case of the myosin heavy chain, the adult forms are not detected as major components of fetal muscle or cultured muscle cells²⁵⁻²⁷. To characterize the developmental changes in more detail, we have investigated the types of myosin heavy chains found at various times after birth in rat skeletal muscle destined to become the fast-contracting type. We used various approaches, including polypeptide mapping, complement fixation, immunocytochemistry, gel electrophoresis of native myosins, and the study of synthetic myosin thick filaments by electron microscopy. Our results provide evidence for the sequential appearance of at least three heavy-chain isozymes during muscle development.

Polypeptide mapping of the myosin heavy chains

The large size of the myosin heavy chain (200,000 M_r) allows it to be cleaved into many polypeptide fragments which are conveniently analysed by standard one- or two-dimensional gel electrophoresis (ref. 27 and refs therein). However, partial proteolytic cleavage of native myosin is influenced by divalent cations²⁸, by phosphorylation of the LC2 light chain²⁹, and by interaction with actin³⁰ and presumably other proteins. It is thus important to denature the myosin preparations before partial proteolysis to minimize influences other than that of the primary structure on the cleavage of the heavy chain. Proteolysis of SDS-denatured myosin suggests that neither the presence of the light chains nor the presence of another myosin species

influences the cleavage of a given heavy-chain type (ref. 27 and S.M.S. and R.G.W., in preparation). This approach has therefore been used to examine the myosin heavy chains present in rat muscle development.

Figure 1 (upper) shows the heavy-chain degradation pattern produced by chymotryptic treatment of various myosins from cultured cells (L6) or fetal, newborn and adult muscle tissue. From about 19 days after birth, the myosin heavy-chain cleavage pattern is indistinguishable from that shown by adult fast myosin. As described previously²⁷, a distinct embryonic myosin heavy chain (MHC_{emb}) can be found in cultured muscle cells and fetal muscle tissue. However, during the first two weeks after birth another heavy chain isozyme appears which differs in its degradation pattern from both the embryonic form and the adult fast and slow forms. We will refer to this isozyme appearing in the neonatal period as MHC_{neo} .

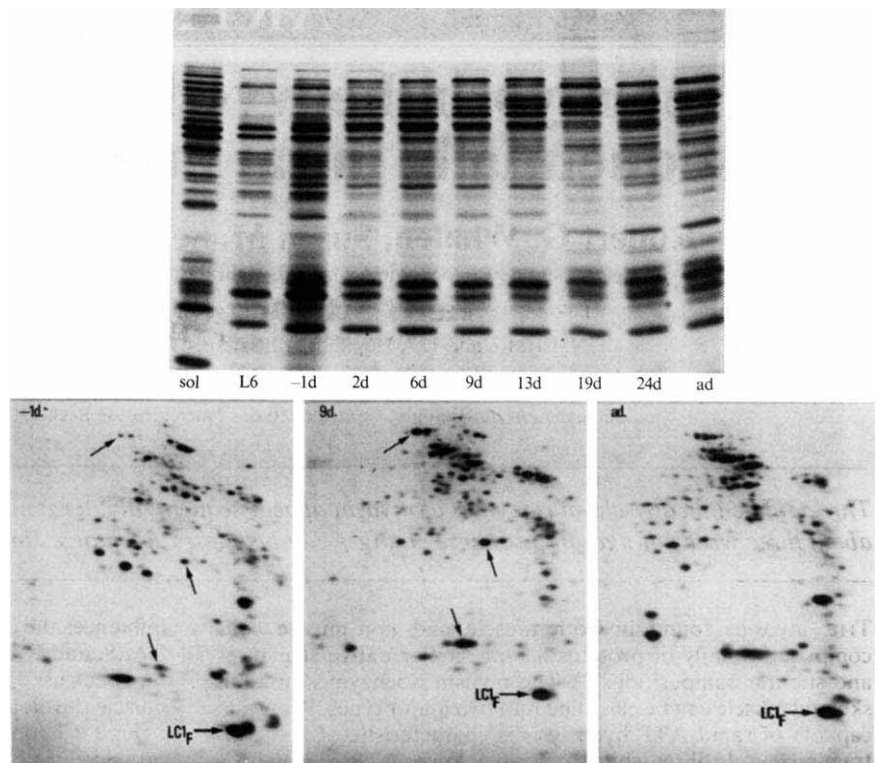
The cleavage pattern produced by the neonatal myosin preparations is unique, as the two-dimensional gel analysis of the chymotryptic polypeptides illustrates (Fig. 1, lower half). This high-resolution analysis shows that the cleavage pattern of neonatal heavy chain is qualitatively different from that of either MHC_{emb} or fast myosin heavy chain (MHC_F). Some characteristic MHC_{neo} polypeptides are shown by arrows in Fig. 1 (lower half). The myosin from fetal tissue (-1 day) contains some MHC_{neo} (arrows; see also Fig. 4). The two-dimensional polypeptide map of MHC_{neo} bears no resemblance to that of the adult slow heavy chain (compare with Fig. 3 of ref. 27). The cleavage pattern of MHC_{neo} is unlike that of any other adult skeletal or cardiac muscle myosin that we have examined nor is it homologous to non-muscle heavy chain (S.M.S. and R.G.W., unpublished observation).

Immunological studies on developmental isozymes of myosin

Complement fixation has been used as a means of measuring the extent of cross-reactivity of a given antibody with different myosins^{27,31-35}. Previous work has shown that the myosin subfragment, heavy meromyosin (HMM), denatured with SDS before injection, produces antibodies with considerable muscle-type specificity. This specificity is greater than when the HMM is injected in its native state^{27,35}. Thus antibodies to SDS-denatured HMM prepared from 8-day old rats were raised in guinea pigs.

In our experiments we used antisera from two different guinea pigs. These anti-neonatal myosin sera react predominantly with the heavy-chain component; this was verified by testing the sera against myosin or crude myosin-containing extracts after separation by SDS-gel electrophoresis and transfer to nitrocel-

Fig. 1 Polypeptide mapping of various myosins. Myosins were prepared as previously described²⁴, the final step being purification on a Sepharose 2B column in the presence of ATP. Myosin was prepared from the adult soleus muscle (sol) as an example of slow myosin, and from L6 cells as an example of a myosin containing the embryonic heavy chain (see text). The remaining myosins were prepared from the hind leg muscles of adult rats (ad), the soleus muscle being deliberately excluded, or from rats at various stages of development (day -1 is 20 days of gestation; the other numbers indicate days after birth). The proportion of slow myosin in the adult hind leg muscle preparations did not exceed 5%, as determined by electrophoretic analysis of native myosin (see Fig. 4 legend). In the upper part of the figure, analysis was carried out on a one-dimensional SDS gel composed of 12.5% (w/v) acrylamide and 0.1% (w/v) bisacrylamide. Myosins (0.4 mg ml^{-1}) were digested with chymotrypsin ($108 \mu\text{g ml}^{-1}$) for 30 min at 37°C as described previously²⁷, and $16 \mu\text{g}$ of each degraded myosin was loaded on to the gel. In the lower part of the figure, $60 \mu\text{g}$ of chymotryptic cleavage products were analysed on two-dimensional gels. For these experiments, the concentration of myosin was 1.2 mg ml^{-1} and that of chymotrypsin $300 \mu\text{g ml}^{-1}$. The ampholytes in the isoelectric focusing gel were composed of 1.6% (w/v), pH 5–8 and 0.4% (w/v), pH 3–10, and the second dimension slab gel was 12.5% (w/v) acrylamide and 0.1% (w/v) bisacrylamide. Two-dimensional gel electrophoresis was carried out as in ref. 24 with the modifications described in ref. 51. The position of the highest molecular weight light chain, LC1_F , is shown for reference as the extent of migration varied slightly among the different samples. Unlabelled arrows indicate characteristic polypeptides of the neonatal heavy chain which are also present in small quantities in the -1 day myosin sample.



lulose paper³⁶ (A.-M. Lompré and K. Schwartz, unpublished results). The myosins to be used as test antigens in the complement fixation reaction were purified on Sepharose 2B columns²⁴, denatured with SDS and mercaptoethanol by heating, and the excess SDS removed as described previously³². These myosins were reacted with the antisera at dilutions chosen to give ~60–70% complement fixation with the homologous antigen (myosin from 10-day old animals). When the antisera were tested with myosin from adult fast muscle, fetal muscle tissue or from L6 cells, the amount of complement fixed was considerably less than in the case of the neonatal myosin (Fig. 2). This result illustrates that there are quantitative antigenic differences between the various myosins, although they share some common determinants. The absence of a reaction for L6 myosin at the dilutions shown does not indicate a total lack of cross-reactivity; if the neonatal myosin antibodies are simply concentrated then a positive reaction can be obtained for the L6 myosin which differs quantitatively from that given by the homologous antigen (result not shown; see also ref. 27). The myosin from fetal muscle tissue gives greater complement fixation than does L6 myosin due to the presence of MHC_{neo} in the tissue preparations (see Figs 1, 4).

These results show that neonatal myosin differs immunologically from both adult myosin and the embryonic form found in fetal muscle tissue and L6 cells. Together with previous results which show immunological differences between L6 and adult myosin²⁷, this approach distinguishes between the three myosins containing MHC_{emb} , MHC_{neo} and MHC_F .

Antibodies to adult fast and slow myosin were also used in indirect immunofluorescence assays on sections of the gastrocnemius muscle of 7–8-day old rats. Most of the fibres in such muscles are stained when reacted with the antibody to adult fast myosin (Fig. 3a). When this antibody is absorbed with myosin extracted from neonatal muscle (see Fig. 3 legend for details), the fluorescence reaction with the neonatal muscle sections is diminished as expected (Fig. 3b). When used at the same dilution, this absorbed antibody will still react with the fast fibres

of the adult diaphragm (Fig. 3c). These results show that the unabsorbed anti-fast myosin antibody cross-reacts with neonatal myosin but that these cross-reacting antibodies can be removed without totally abolishing the reaction with adult fast myosin. An antibody to adult cardiac myosin which cross-reacts extensively with slow-twitch skeletal myosin^{27,35} was used as a probe for slow myosin-containing fibres. Few fibres stained brightly (Fig. 3d); the proportion of positive fibres varied according to the region of the muscle examined. These immunocytochemical results thus completely agree with the

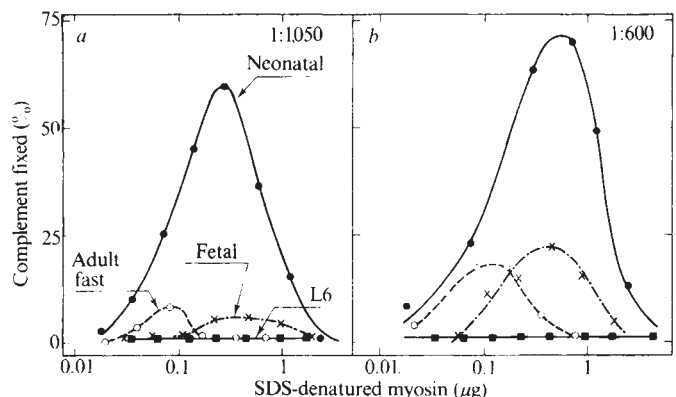


Fig. 2 Complement fixation reactions using antisera to neonatal myosin. Heavy meromyosin (HMM) was prepared from the myosin extracted from the hind leg muscle of 8-day old rats³³. The HMM was denatured with SDS and the excess SDS removed on a G-10 column as described elsewhere³² before injection into five guinea pigs. Four animals developed antibodies; the results shown here are for two of these antisera. The test antigens were prepared from adult muscle (adult fast, ○), muscle of 10-day old animals (neonatal, ●), muscle from animals at 20 days of gestation (fetal, ×) and from fused cultures of L6 cells (L6, ■). Each test antigen was denatured with SDS and the excess SDS removed on a G-10 column³² before reaction in the complement fixation test^{33,35}.

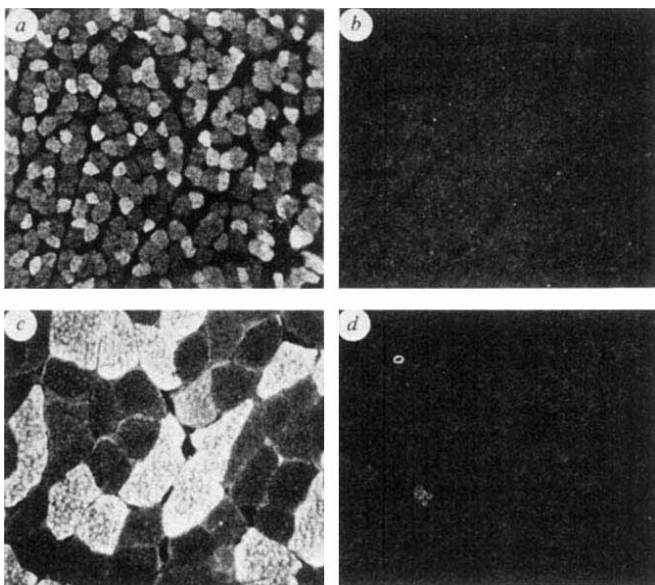


Fig. 3 Immunocytochemical reactions of neonatal and adult muscles. Indirect immunofluorescence was carried out using antibodies prepared in guinea pigs. A fluorescein-labelled goat anti-guinea pig immunoglobulin antiserum (Nordic) was used as the second antibody. In *a*, an antibody to adult fast myosin³⁵ was reacted at a dilution of 1:100 with a section of 7-day gastrocnemius muscle. The anti-fast antibody was also absorbed with myosin prepared from neonatal muscle by first mixing the myosin and antiserum in 0.6 M NaCl and then dialysing to low ionic strength to precipitate the myosin. In *b*, this absorbed antibody was reacted at a dilution of 1:40 with the 7-day gastrocnemius muscle. In *c*, the 1:40 diluted absorbed antibody was reacted with a section of adult diaphragm; it reacts with those fibres that react as fast fibres in histochemical staining (results not shown). *d*, The 7-day gastrocnemius muscle was stained with an antibody to adult cardiac myosin^{27,35}.

polypeptide mapping and complement fixation experiments in that they show differences between neonatal and adult myosins.

Electrophoresis of native myosins

The various myosin preparations were further investigated by gel electrophoresis in native conditions^{37,38}; L6 myosin (containing MHC_{emb}) gave only one band (Fig. 4). This same band is found in extracts of fetal tissue at 3 days before birth. Two days later, and in the first few days after birth, another band is present which is distinct from that containing MHC_{emb} (see also the results for the mixture of L6 myosin and a 10-day preparation); this corresponds to the appearance of myosin containing MHC_{neo} (Fig. 1). In the second week after birth, three bands are seen for neonatal myosin; the presence of three bands at this time is probably due to the formation of isoenzymes based on light chain content^{25,39}. From about 14 days onward, the adult fast myosin bands can be seen. The adult slow myosin isozymes are clearly separated from the embryonic, neonatal and adult fast forms (Fig. 4). This analysis confirms the time course of the developmental sequence observed using the polypeptide mapping approach (Fig. 1).

Formation of synthetic myosin filaments

The size and structure of synthetic thick filaments formed from adult fast skeletal myosin depends critically on the relative concentrations of ATP and Mg²⁺ in the dilution buffer⁴⁰. We have therefore compared the effect of these parameters on the formation of synthetic filaments obtained from myosins containing the embryonic, neonatal and adult fast and slow heavy chains.

Synthetic filaments were formed by controlled dilution of myosin and were examined by electron microscopy as previously described⁴⁰. In these experiments, the concentration of ATP in the diluting buffer was kept constant at 0.1 mM and the

Mg²⁺ concentration varied between 0.1 and 10 mM. Electron micrographs of the various types of filaments obtained from neonatal myosin are shown in Fig. 5*a-c*. Depending on the concentration of Mg²⁺, three types of filaments were observed. At intermediate concentrations bipolar filaments with tapering ends and constant diameters of 15–20 nm were found (Fig. 5*a*). This type of filament is structurally similar to natural ones isolated from adult fast skeletal muscle^{40,41}. At high concentrations of Mg²⁺, thicker spindle-shaped filaments up to 50–60 nm in diameter were formed (Fig. 5*b*). At low concentrations of Mg²⁺, thin branched structures, ~5–7 nm wide were observed (Fig. 5*c*). The neonatal myosin filament preparations thus behave qualitatively in the same way as those of adult fast myosin⁴⁰.

The concentration range over which these types of filaments were found differed for the several myosins tested (results shown schematically in Fig. 5, lower left). Both fast and slow adult myosins form bipolar filaments with constant diameters over a similar, large concentration range (0.5–10 mM Mg²⁺). The Mg²⁺ values at which such bipolar filaments are found is narrow for neonatal myosin and is particularly restricted with L6 myosin. In the latter case, homogeneous populations of bipolar filaments with constant diameters were rarely obtained. Furthermore, the lengths of the bipolar filaments formed in the presence of 2 mM Mg²⁺ differed for the various myosins (see histogram, Fig. 5, lower right). Both adult fast and slow myosin formed very long filaments (5–7 μ m), longer in fact than natural filaments from fast muscle, which are 1.6 μ m long⁴¹. Neonatal myosin formed filaments of intermediate length (2–4 μ m) and L6 myosin only very short ones (<1 μ m).

The formation of myosin thick filaments is due to the aggregation of the heavy-chain subunit, in particular the C-terminal α -helical portion. It has been suggested that the LC2 light chain might influence filament formation^{42,43}. In adult fast, neonatal and L6 myosins, the LC2 light chain seems to be the same (ref. 24 and R.G.W., results not shown). Thus the differences in filament formation between these three myosins are probably attributable to differences among the heavy chains, in agreement with our biochemical and immunological results.

Relationship to previous studies

We have demonstrated that development of rat skeletal muscle tissue is characterized by the presence of specific myosin isozymes differing from both the fast and slow adult forms. These results are consistent with several previous studies. Earlier work by Trayer and Perry¹⁰ has shown that the Ca²⁺-ATPase activity of myosin changes with development of the muscles of several animals, including rats. In addition, Drachman and Johnston⁹ have shown that actomyosin ATPase activity is low in the neonatal rat EDL (extensor digitorum longus) muscle until 5 days after birth. After this time the activity increases, reaching adult values by ~20 days. These changes in ATPase activities

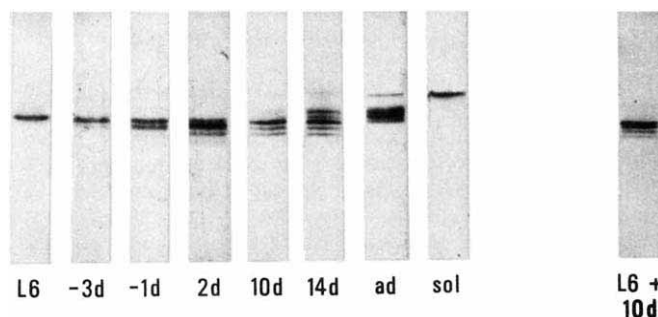


Fig. 4 Electrophoresis of native myosins in non-dissociating conditions. Myosin samples (1–3 μ g) were run for 16 h at 14 V cm⁻¹ and 2 °C on cylindrical gels (5 × 60 mm) which consisted of 3.88% (w/v) acrylamide and 0.12% (w/v) bisacrylamide and contained 20 mM sodium pyrophosphate (pH 8.5), 10% (v/v) glycerol, 1 mM EDTA and 0.01% (v/v) 2-mercaptoethanol. Migration was from top (cathode) to bottom (anode). Abbreviations as in Fig. 1.

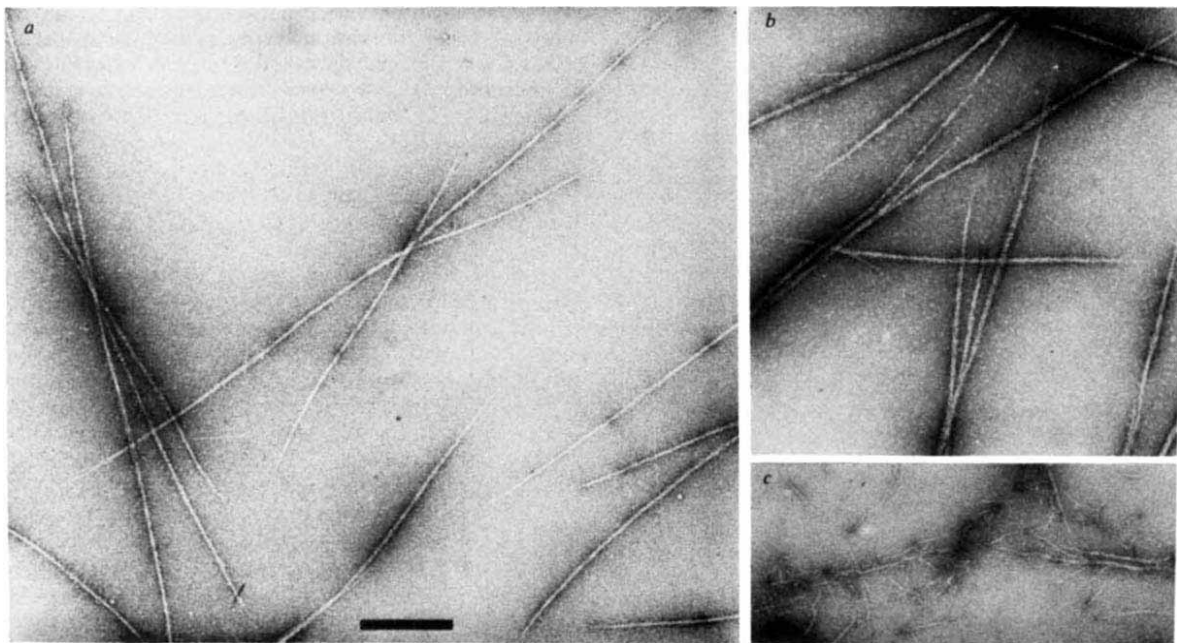
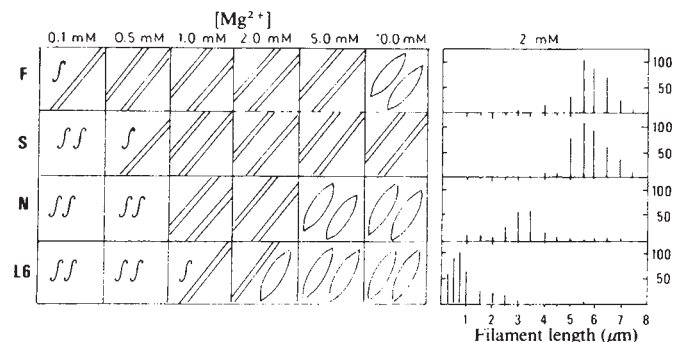


Fig. 5 Electron microscopy of synthetic myosin filaments. The upper part of the figure shows representative micrographs illustrating the overall appearance of filaments formed from neonatal myosin (10-day old muscle) in the presence of 0.1 mM ATP. The Mg^{2+} concentration in the polymerization medium was: a, 2 mM Mg^{2+} ; b, 10 mM Mg^{2+} and c, 0.1 mM Mg^{2+} . Filaments were formed by controlled dilution and prepared for electron microscopic examination as previously described⁴⁰. The bar in a represents 0.5 μ m. The lower left part of the figure shows a schematic representation of the electron microscopic observations of the filaments formed from several types of myosins: adult fast (F), adult slow (S), neonatal (N) and embryonic myosin from fused L6 cells. Polymerization was carried out in the presence of 0.1 mM ATP and the Mg^{2+} concentration was varied over the values indicated. The parallel lines indicate the presence of long, bipolar filaments of 15–20 nm diameter (as in a; see text). The leaf-shaped symbols indicate the presence of filaments of thicker diameter (as in b). The curved lines indicate the presence of short, branched structures (as in c). Two identical symbols in the same square indicate that the filament population was homogeneous; two different symbols indicate that the sample was apparently a mixture of filament types. The lower right part of the figure is a histogram showing the distribution of filament lengths (in μ m) observed at 2 mM Mg^{2+} for the four different myosins. In all the experiments shown here, the preparation of the myosin and the electron microscopy did not exceed 24 h to minimize any phenomena due to ageing of the myosin as noted previously⁴². A shortened protocol was therefore used to prepare the myosin. The muscle tissue was homogenized in a Polytron homogenizer in three volumes of a buffer containing 0.5 M NaCl, 0.05 M Tris-HCl (pH 6.8), 10 mM $MgCl_2$ and 3 mM ATP. After extraction for 20 min at 0°C, the sample was centrifuged at 15,000g for 20 min. More ATP was added to the supernatant to give a final concentration of 6 mM, and the sample was centrifuged at 400,000g for 1 h or more to remove polymeric actin. The resultant supernatant was dialysed against a large volume of 40 mM NaCl, 30 mM Tris-HCl (pH 6.8). After ~3 h the precipitated myosin was collected by centrifugation and resuspended in 0.5 M NaCl, 20 mM Tris-HCl (pH 6.8). The myosin was stored on ice for several hours until the start of the electron microscopy experiments.



may be a manifestation of the transitions taking place among different myosin isozymes, as they occur in rats at about the same time as the observed transition from neonatal to adult myosin.

Trayer and Perry¹⁰ have also shown that changes in ATPase occur somewhat earlier in rabbit than in rat. Using polypeptide mapping, we have found that the predominant myosin heavy chain in newborn rabbits is homologous to the rat neonatal form (results not shown). Migration of the rat neonatal isozymes in native electrophoresis is also very similar to that of newborn rabbit myosin²⁵. The neonatal form of rabbit myosin persists until at least 10–15 days after birth (results not shown). This observation can be related to studies of amino acid sequence: Huszar⁴⁴ found that the myosin from 3-week old rabbits contains two different, yet homologous, histidine-containing tridecapeptides whereas adult myosin retains only one. From our results, the additional sequence found at 3 weeks post-natal probably corresponds to that of rabbit neonatal myosin and not embryonic or adult slow myosin. Both the sequence studies and the results of polypeptide mapping show that the isozyme transitions involve heavy chains that differ in primary structure.

Our results are consistent with previous immunocytochemical studies on developing rat muscle^{14,16} which used antibodies directed against the adult fast and slow myosins. It is likely that these antibodies cross-reacted with MHC_{emb} , MHC_{neo} or both. The use of antibodies to native myosin has demonstrated considerable antigenic homology among adult fast and slow as well as embryonic myosin heavy chains^{27,34}. Moreover the adult anti-fast myosin antibody used here (Fig. 3) clearly cross-reacts with neonatal myosin. However, it can be noted that Gauthier *et al.*¹⁴ found that the adult pattern of antibody staining is established by the third week post-natal. This timing correlates well with the disappearance of the neonatal isozyme.

The conflicting results of immunocytochemical studies on developing chicken muscle^{13,15,19,21} may also be due to differing degrees of cross-reactivity of the antibodies with myosin types analogous to the rat embryonic and neonatal forms. It has been claimed²¹ that the myosin heavy chain of cultured chicken cells is identical to the adult protein, based on results of Ouchterlony double diffusion. It was found that if a mixture of radioactively-labelled, cultured myosin and adult fast myosin was diffused against antibodies to fast myosin then both proteins were found

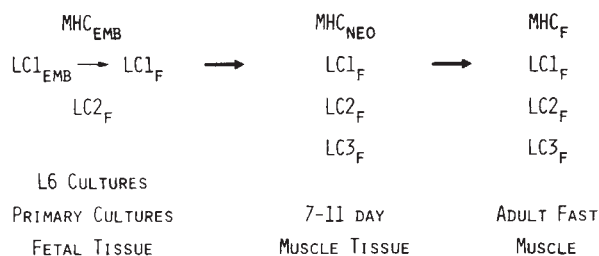


Fig. 6 Schematic representation of the myosin subunits present at various times during fast muscle development. This representation is meant to illustrate concisely the predominant polypeptides present in a given situation; it does not show explicitly the stoichiometry of the native molecule nor does it indicate presumably minor combinations of heavy and light chains (such as LC1_{emb} with MHC_{neo}).

in the same precipitation line²¹. Such a result is precisely that expected if the culture myosin is different from the adult fast protein but simple shares some antigenic determinants with the latter. Polypeptide mapping studies provide evidence for a distinct developmental form of myosin heavy chain in chickens (refs 26, 45 and S.M.S. and R.G.W., unpublished observations). Thus there may be parallels between avian and mammalian muscle development concerning the myosin heavy-chain isozymes.

Innervation and myosin isozyme transitions in muscle development

A schematic representation of the myosin transitions occurring in rat muscle development is given in Fig. 6. Our data suggest that the first heavy-chain isozyme to appear after muscle cell fusion is that designated MHC_{emb}, based on results with cultured myotubes and fetal muscle tissue^{27,46}. The light-chain subunits associated with this heavy chain evolve as cultured myotubes then mature⁴⁶. The predominant myosin heavy-chain form at different stages of development is either the embryonic or the neonatal isozyme (Fig. 6). We cannot, however, rule out the presence of small amounts of the adult fast and slow types at these stages. Immunocytochemical studies of fetal rat muscles suggest the presence of a major fibre population containing slow myosin¹⁶. Whether this result is simply due to a cross-reaction with embryonic myosin is unknown. The precise sequence of appearance of these four isozymes within individual fibres can be determined when antibodies specific to each type become available. It is possible, for example, that different fibre types evolve differently¹⁶ and that MHC_{emb} may be a component of one type and MHC_{neo} a component of another.

Activity or trophic influences of the nerve are important in determining the phenotype of the muscle in the adult animal, and similar mechanisms might operate during development^{5,6}. Muscle fibres of the newborn rat are in contact with several nerve axons⁴⁷. This polyneuronal innervation regresses so that by ~3 weeks after birth only one axon remains per muscle fibre. Results which suggest that both fast and slow adult myosins are present in the early developmental stages¹⁴ could be taken to indicate that a switching off of one or the other myosin type occurs in response to the establishment of the adult configuration of the neuromuscular junction. It has also been suggested²⁰ that only fast myosin is present in developing muscle fibres and that the induction of slow myosin can occur as a specific nerve impulse pattern is established during the regression of polyneuronal innervation.

However, our results have shown that the predominant myosin heavy-chain isozymes in developing muscle are neither adult fast myosin nor a mixture of the adult fast and slow types. One possibility is that the absence of well defined nerve activity or trophic pattern at these stages is responsible for the absence of adult myosin types. Embryonic myosin is synthesized by cultured myotubes in the absence of innervation^{27,46}. Whether the neonatal heavy-chain form is actively induced as a consequence of polyneuronal innervation, or whether it can be synthesized by the muscle fibre in the absence of any nervous input is unknown.

This is the first demonstration of three myosin heavy-chain isozymes occurring sequentially in fast muscle development. There have been other demonstrations of the sequential appearance of proteins of related structure and function, for example, the embryonic, fetal and adult forms of globin⁴⁸, or α -fetoprotein and serum albumin⁴⁹. The class switch of immunoglobulin molecules in the maturing lymphocyte is also an example of the sequential expression of protein isoforms. This immunoglobulin switching occurs via deletions of the DNA coding for the constant-region domains of the antibody molecule (see ref. 50). Myosin is a protein also composed of several clearly different domains¹. Whether the isozymic transitions of the myosin heavy chain reported here are related to gene deletions and fusions can be tested at the protein level, by comparing the various subfragments of the different isozymes, and at the DNA level, using cloned gene fragments.

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- Clarke, M. & Spudis, J. A. *Rev. Biochem.* **46**, 797–822 (1977).
- Burridge, K. & Bray, D. *J. molec. Biol.* **99**, 1–14 (1975).
- Chi, J. C., Fellini, S. A. & Holtzer, H. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4999–5003 (1975).
- Scordilis, S. & Adelstein, R. A. *Nature* **268**, 558–560 (1977).
- Pette, D. (ed.) *Plasticity of Muscle* (Walter de Gruyter, Berlin, 1980).
- Salmons, S. & Henrikson, J. *Muscle & Nerve* **4**, 94–105 (1981).
- Buller, A. J., Eccles, J. C. & Eccles, R. M. *J. Physiol., Lond.* **150**, 399–416 (1960).
- Close, R. *J. Physiol., Lond.* **173**, 74–95 (1964).
- Drachman, D. B. & Johnston, D. M. *J. Physiol., Lond.* **234**, 29–42 (1973).
- Trayer, I. P. & Perry, S. V. *Biochem. Z.* **345**, 87–100 (1966).
- Brevet, A. & Whalen, R. G. *Biochimie* **60**, 459–466 (1978).
- John, H. A. *FEBS Lett.* **64**, 116–121 (1976).
- Masaki, T. & Yoshizaki, C. *J. Biochem., Tokyo* **76**, 123–131 (1974).
- Gauthier, G. F., Lowey, S. & Hobbs, A. W. *Nature* **274**, 25–29 (1978).
- Cantini, M., Sartore, S. & Schiaffino, S. *J. Cell Biol.* **85**, 903–909 (1980).
- Kelly, A. M. & Rubinstein, N. A. *Nature* **288**, 266–269 (1980).
- Dow, J. & Stracher, A. *Proc. natn. Acad. Sci. U.S.A.* **68**, 1107–1110 (1971).
- Streter, F. A., Balint, M. & Gergely, J. *Dev Biol.* **46**, 317–325 (1975).
- Rubinstein, N. A., Pepe, F. A. & Holtzer, H. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4524–4527 (1977).
- Rubinstein, N. A. & Kelly, A. M. *Dev Biol.* **62**, 473–485 (1978).
- Rubinstein, N. A. & Holtzer, H. *Nature* **280**, 323–325 (1979).
- Whalen, R. G. *Plasticity of Muscle* (ed. Pette, D.) 177–191 (Walter de Gruyter, Berlin, 1980).
- Whalen, R. G. *Adv. physiol. Sci.* **5**, 63–69 (1981).
- Whalen, R. G., Butler-Browne, G. S. & Gros, F. *J. molec. Biol.* **126**, 415–431 (1978).
- Hoh, J. F. Y. & Yeoh, G. P. S. *Nature* **280**, 321–323 (1979).
- Rushbrook, J. I. & Stracher, A. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4331–4334 (1979).
- Whalen, R. G., Schwartz, K., Bouveret, P., Sell, S. M. & Gros, F. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5197–5201 (1979).
- Weeds, A. G. & Pope, B. *J. molec. Biol.* **111**, 129–157 (1977).
- Ritz-Gold, C. J., Cooke, R., Blumenthal, D. K. & Stull, J. T. *Biochem. biophys. Res. Commun.* **93**, 209–214 (1980).
- Mornet, D., Pantel, P., Audemard, E. & Kassab, R. *Biochem. Biophys. Res. Commun.* **89**, 925–932 (1979).
- Bruggmann, S. & Jenny, E. *Biochim. biophys. Acta* **412**, 39–50 (1975).
- Lompré, A.-M., Bouveret, P., Leger, J. & Schwartz, K. *J. immunol. Methods* **28**, 143–148 (1979).
- Schwartz, K., Bouveret, P., Sebag, C., Leger, J. & Swynghedauw, B. *Biochim. biophys. Acta* **42**, 24–36 (1977).
- Schwartz, K., Bouveret, P. & Sebag, C. *FEBS Lett.* **87**, 99–102 (1978).
- Schwartz, K., Lompré, A.-M., Bouveret, P., Wisniewsky, C. & Swynghedauw, B. *Eur. J. Biochem.* **104**, 341–346 (1980).
- Bowen, B., Steinberg, J., Laemmli, U. K. & Weintraub, H. *Nucleic Acids Res.* **8**, 1–20 (1979).
- Hoh, J. F. Y., McGrath, P. A. & White, R. I. *Biochem. J.* **157**, 87–95 (1976).
- d'Albis, A., Pantaloni, C. & Bechet, J.-J. *Eur. J. Biochem.* **99**, 261–272 (1979).
- Lowey, S., Benfield, P. A., Silberstein, L. & Lang, L. M. *Nature* **282**, 522–524 (1979).
- Pinset-Härström, I. & Truffly, J. *J. molec. Biol.* **134**, 173–188 (1979).
- Huxley, H. E. *J. molec. Biol.* **7**, 281–308 (1963).
- Pinset-Härström, I. & Whalen, R. G. *J. molec. Biol.* **134**, 189–197 (1979).
- Scholey, J. M., Taylor, K. A. & Kendrick-Jones, J. *Nature* **287**, 233–235 (1980).
- Huszar, G. *Nature new Biol.* **240**, 260–264 (1972).
- Benfield, P. A., LeBlanc, D. D. & Lowey, S. *Fedn Proc.* **39**, 2169 (Abstr. 2966) (1980).
- Whalen, R. G., Butler-Browne, G. S., Sell, S. & Gros, F. *Biochimie* **61**, 625–632 (1979).
- Brown, M. C., Jansen, J. K. S. & Van Essen, D. C. *J. Physiol., Lond.* **261**, 387–425 (1976).
- Weatherall, D. J. & Clegg, J. B. *Cell* **16**, 467–479 (1979).
- Law, S. W. & Dugaiczky, A. *Nature* **291**, 201–205 (1981).
- Molgaard, H. V. *Nature* **286**, 657–659 (1980).
- Whalen, R. G. & Sell, S. M. *Nature* **286**, 731–733 (1980).

LETTERS TO NATURE

Distinguishing between a white dwarf and a neutron star in an X-ray binary

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It is proposed that the single most useful clue to the nature of the accreting compact star in an X-ray binary is its ratio of X-ray to visual luminosity L_X/L_V (2–10 keV)/ L_V . Present data indicate that typical L_X/L_V ratios are ~ 4 for white dwarfs, and ~ 5000 for neutron stars. These numbers are straightforward consequences of the energetics of accretion, and are approximately independent of the distance, stellar mass, and mass transfer rate. The data suggest that the ratios are also not very sensitive to any of the other system parameters, except the radius of the compact star.

Remarkably, there is still no general and reliable method for determining whether the accreting component in a galactic X-ray binary is a white dwarf or neutron star. Davidson and Ostriker¹ and Lamb *et al.*² argued that the pulsation periods of Cen X-3 and Her X-1 are too short to represent white dwarf pulsation or rotation periods. Lamb *et al.*² and Rappaport and Joss³ used the observed spin-up time scales in X-ray pulsars to establish the presence of a neutron star in most or all of these systems. Joss⁴ identified X-ray bursters as neutron stars, and Kylafis and Lamb⁵ suggested that all sources with $L_X \geq 5 \times 10^{36}$ erg s⁻¹ contain neutron stars. None of these criteria are generally applicable, however, as most sources do not pulse, burst, or reveal their distances. The X-ray spectrum was once thought to be a useful discriminant, when the first detection of an accreting white dwarf showed a very soft spectrum⁶. But subsequent observation has shown that accreting white dwarfs are generally quite hard sources, with bremsstrahlung temperatures of ~ 10 –30 keV (ref. 7). I propose here that the most sensitive and useful indicator of the identity of the compact star is its ratio of X-ray to visual luminosity L_X/L_V . This is easily measured; approximately independent of the distance, stellar mass and mass transfer rate; easily understood in terms of the underlying energetics of accretion; and seems to give the right answer. Figure 1 shows all available data on optically identified compact X-ray sources. These divide neatly into two classes.

(1) The filled squares in Fig. 1 are sources discovered by scanning X-ray telescopes (hence 'X-ray selected'), and represent a nearly complete sample down to a flux level of $F_X(2-10 \text{ keV}) \approx 4 \times 10^{-11}$ erg cm⁻² s⁻¹. Bradt *et al.*⁸ presented data on $L_X(2-10 \text{ keV})/L_V$ for 54 sources, and seven subsequent identifications have been added: 2A0311–227 ($L_X/L_V = 5.5$) (refs 9, 10), 2A0526–328 (1.6) (ref. 11), H2252–035 (3.2) (ref. 12), V1223 Sgr (2.2) (ref. 13), U Gem (1.4) (ref. 14), GK Per (5.2) (refs 15, 16) and 4U1556–60 (2,300) (ref. 17). Although Bradt *et al.*⁸ presented their data in terms of 'optical luminosity' (after applying the best available bolometric correction), we have used instead the visual flux and apply only their correction for visual absorption A_V . Thus we avoid working with large and uncertain bolometric corrections. No correction to the 2–10 keV X-ray flux is necessary, because the X-ray cutoff energies are known to be outside this bandpass. To determine the L_X/L_V ratio of the accreting component (including an accretion disk if present), we must subtract the visual luminosity of the secondary. Where both components are visible (HZ Her, 4U2129+47, H2252–035, Cyg X-2, SS Cyg, GK Per, 2S0921–63, possibly SMC X-1), this can be done with reasonable precision; but in 23 of the 61 stars, the luminosity of the secondary is completely dominant at visual wavelengths, and hence only a lower limit to L_X/L_V can be obtained. Consequently, we exclude these 23 stars from Fig. 1. The remaining 38 stars are shown as the filled squares in Fig. 1.

The open squares in Fig. 1 are Einstein Observatory imaging

proportional counter (IPC) measurements of 25 additional optically selected cataclysmic variables, drawn from available lists (refs 7, 18, unpublished data, and P. Szkody, personal communication). We use the IPC counting rate in the 0.55–4.0 keV energy channels and the visual magnitude m_V as a measure of L_X/L_V . Because eight of the X-ray-selected objects are cataclysmic variables which have been observed with the IPC, we may calibrate this ratio by requiring that the Einstein L_X/L_V equals the ratio deduced from the data of Bradt *et al.*⁸. This converts roughly to: $L_X/L_V = 1$ for $m_V = 13.7$, IPC count rate = 0.22 c.p.s. (counts per s). This conversion should be fairly reliable as all these objects are nearby (~ 100 pc, rendering interstellar absorption unimportant), have similar *UBV* colours, and have similarly hard X-ray spectra. In addition to the 33 positive detections (including the eight X-ray-selected objects), there are six objects not detected, corresponding to $L_X/L_V \leq 0.1$; these are not shown in Fig. 1.

A bimodal distribution in the L_X/L_V ratios is visible in Fig. 1: the sources cluster around mean values of $L_X/L_V = 4$ and 4,900. Because there are two known stable configurations for compact stars with radii differing by a factor of $\sim 10^3$, the neutron-star and white-dwarf population of the two classes should be investigated. The existence of neutron stars in systems containing X-ray pulsars with short spin-up times, and neutron stars or black holes in systems containing X-ray bursters or with $L_X \geq 5 \times 10^{36}$ erg s⁻¹ can be certified, as can the existence of white dwarfs in classical novae, dwarf novae, AM Herculis stars, and X-ray pulsars with long spin-up times. To avoid optical selection effects only X-ray selected objects are considered. All certified white dwarfs and neutron stars are labelled W or N respectively in Fig. 1. In the group near $L_X/L_V = 5,000$, we find 12 neutron stars, no white dwarfs, and 17 uncertain cases. In the group near $L_X/L_V = 4$, we find no neutron stars, seven white dwarfs, and two uncertain cases. The natural inference is that accreting neutron stars typically have $L_X/L_V \sim 5,000$, white dwarfs typically have $L_X/L_V \sim 4$, and the lack of overlap between the two classes emphasizes the value of L_X/L_V as a signature of the presence of a white dwarf or neutron star. The Einstein observations of cataclysmic variables (which are known to contain white dwarfs) confirm that our nine objects near $L_X/L_V = 4$ are not aberrant cases, but are fairly typical of accreting white dwarfs (with high L_X/L_V objects preferentially detected in X-ray surveys, as would be expected).

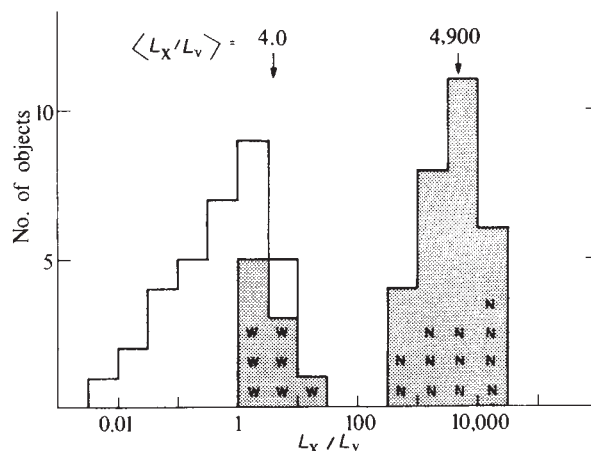


Fig. 1 Distribution of L_X/L_V ratios for the accreting component of optically identified compact X-ray sources. $\blacksquare$, Sources identified from scanning X-ray telescopes; $\square$, Einstein observations of optically selected cataclysmic variables (the X-ray and optical measurements during quiescence were used for dwarf novae). The average values of L_X/L_V are indicated for the group near $L_X/L_V = 1$ and $= 5,000$. Systems known to contain white dwarfs and neutron stars are labelled W and N respectively.

This result is easy to understand in terms of the energetics of disk accretion, as most of the optical light emerges from large radii in the disk (where there is no dependence on the nature of the compact star), but the X rays emerge from gravitational potential wells which differ in depth by a factor of $\sim 1,000$. For a self-luminous steady-state accretion disk¹⁹ surrounding a $1 M_{\odot}$ neutron star, (or black hole, which is for present purposes equivalent to a neutron star), with $\dot{M} = 10^{-10} M_{\odot} \text{ yr}^{-1}$, only 5% of the optical luminosity is produced at radii less than a typical white dwarf radius of $6 \times 10^8 \text{ cm}$. Thus, for accretion rates near $10^{-10} M_{\odot} \text{ yr}^{-1}$, we have approximately

$$L_v = 3 \times 10^{32} \text{ erg s}^{-1} \left(\frac{M_*}{1 M_{\odot}} \right) \left(\frac{\dot{M}}{10^{-10} M_{\odot} \text{ yr}^{-1}} \right) \quad (1)$$

independent of the nature of the compact star. But the bolometric luminosity (L_{bol}) depends on the radius R_* of the compact star:

$$L_{\text{bol}} = 3 \times 10^{33} \text{ erg s}^{-1} \left(\frac{M_*}{1 M_{\odot}} \right) \left(\frac{\dot{M}}{10^{-10} M_{\odot} \text{ yr}^{-1}} \right) \left(\frac{R_*}{6 \times 10^8 \text{ cm}} \right)^{-1} \quad (2)$$

If a fraction k of the accretion energy emerges in hard X rays, then $L_x = k L_{\text{bol}}$, and therefore

$$L_x/L_v = 10 k \left(\frac{R_*}{6 \times 10^8 \text{ cm}} \right)^{-1} \quad (3)$$

Now for both neutron stars and white dwarfs, the observed bremsstrahlung temperatures are in the range 10–30 keV, hence our adopted 2–10 keV window will typically miss $\sim 50\%$ of the X-ray flux. For white dwarfs, a well-formed accretion disk may radiate at optical and UV wavelengths much of the total gravitational energy available, lowering the value of k still further. Reasonable values are therefore $k = 0.5$ for a neutron star and $k = 0.2$ for a white dwarf. Then the expected values of L_x/L_v become 2 and 3,000 for white dwarfs and neutron stars respectively.

Refinements to this plausibility argument—especially the consideration of detailed emission mechanisms—will be worth adding in the future. Two of the stars in the X-ray-selected sample are AM Her stars, in which accretion is thought to proceed by radial infall onto the white dwarf, without the mediation of an accretion disk. Yet their L_x/L_v ratios are typical of the entire class of accreting white dwarfs (4.0 for AM Her, 5.5 for 2A0311–227). Similarly, some fraction of the accreting neutron stars may produce most of their visual luminosity by reprocessing X-rays in the disk, which ought to lower the L_x/L_v ratio from that expected in a pure accretion model. However, there is no obvious signature of the reprocessors in Fig. 1. We can speculate that the AM Her stars will be found to inhabit the high end of the L_x/L_v distribution for accreting white dwarfs, and the reprocessors will prefer the low end of the L_x/L_v distribution for accreting neutron stars. Good candidates for the latter include A0620–00, 2S0921–63, HZ Her, 4U1626–67, 4U1659–49, 4U1822–37, Aql X-1, and 4U1957+11. But the data do not yet demand this segregation. Presumably, the great difference in the depths of white dwarf and neutron star gravitational potential wells overpowers effects arising from the local geometry and the detailed emission mechanisms. Because L_x/L_v is simple to measure and depends strongly only on the radius of the compact star, it seems to be the most useful and reliable indicator of the nature of the compact star.

How will discarding our selection criteria affect Fig. 1? The Einstein observations of cataclysmic variables show that the mean value of L_x/L_v for white dwarfs will decrease to ~ 1 . The mean value for neutron stars will probably increase, as there are many unidentified sources with small error boxes containing only very faint stars (whereas X-ray sources with bright counterparts have probably not been missed). In addition, our deduced values of L_x/L_v must be increased if a significant fraction of the optical light is produced by means other than accretion—a very real possibility for neutron-star sources. Thus it seems likely that the empirical gulf between white dwarfs and

neutron stars in Fig. 1 will remain sufficiently wide to provide an unambiguous discriminant.

The use of the L_x/L_v discriminant should help to classify newly discovered X-ray binaries. Among presently known sources, there are controversies concerning the nature of the compact star in Sco X-1 ($L_x/L_v = 5,000$) (ref. 20), Cyg X-2 (11,000) (ref. 20), 4U1822–37 (300) (ref. 21), 4U2129+47 (1,900) (ref. 22), and H2252–035 (3.2) (refs 23, 24). According to our criterion, the first four should be classified as neutron stars, and the last as a white dwarf. Of course, our criterion does not distinguish neutron stars from black holes. Finally, it should be emphasized that 38% of the identified sources have not been determined, in which the existence of a luminous secondary prevents the use of the L_x/L_v discriminant.

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1. Davidson, K. & Ostriker, J. P. *Astrophys. J.* **179**, 585 (1973).
2. Lamb, F. K., Pethick, C. J. & Pines, D. *Astrophys. J.* **184**, 271 (1973).
3. Rappaport, S. A. & Joss, P. C. *Nature* **266**, 683 (1977).
4. Joss, P. C. *Astrophys. J. Lett.* **225**, L123 (1978).
5. Kyllafis, N. D. & Lamb, D. Q. *Astrophys. J. Lett.* **228**, L105 (1979).
6. Rappaport, S. A., Cash, W. & Doxsey, R. *Astrophys. J. Lett.* **187**, L5 (1974).
7. Cordova, F. A., Mason, K. O. & Nelson, J. E. Preprint (1981).
8. Bradt, H. V., Doxsey, R. E. & Jernigan, J. G. in *COSPAR Symp. Ser. Vol. 3* (eds Baity, W. A. & Peterson, L. E.) 3, Pergamon, New York, 1979).
9. Griffiths, R. E. *et al. Astrophys. J. Lett.* **232**, L27 (1979).
10. Williams, G. *et al. Nature* **281**, 48 (1979).
11. Charles, P., Thorstensen, J., Bowyer, S. & Middleditch, J. *Astrophys. J. Lett.* **231**, L131 (1979).
12. Griffiths, R. E. *et al. Mon. Not. R. astr. Soc.* **193**, 25P (1980).
13. Steiner, J. *et al. Astrophys. J. Lett.* (in the press).
14. Swank, J. S., Boldt, E. A., Holt, S. S., Rothschild, R. E. & Serlemitsos, P. J. *Astrophys. J. Lett.* **226**, L133 (1978).
15. King, A. R., Ricketts, M. J. & Warwick, R. S. *Mon. Not. R. astr. Soc.* **187**, 77P (1979).
16. Forman, W. *et al. Astrophys. J. Suppl.* **38**, 357 (1978).
17. Charles, P. *et al. Bull. Am. astr. Soc.* **11**, 720 (1979).
18. Becker, R. H. & Marshall, F. E. *Astrophys. J. Lett.* (in the press).
19. Pringle, J. E. & Rees, M. J. *Astr. Astrophys.* **22**, 1 (1972).
20. Branduardi, G., Kyllafis, N. D., Lamb, D. Q. & Mason, K. O. *Astrophys. J. Lett.* **235**, L153 (1980).
21. White, N. E. *et al. Astrophys. J.* (in the press).
22. Charles, P. A. & Thorstensen, J. Preprint (1980).
23. Patterson, J. & Price, C. *Astrophys. J. Lett.* **243**, L83 (1981).
24. White, N. E. & Marshall, F. E. Preprint (1981).

Pulsar slow-down epochs

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Theoretical arguments about the progenitors of pulsars suggest that all neutron stars may have essentially the same mass^{1,2} m and the same magnetic moment^{3,4} $\dot{M}$. Direct determinations^{5,6} support this view and give $m \approx 10^{33.5} \text{ g}$ (the Chandrasekhar mass) and $\dot{M} \approx 10^{30.5} \text{ G cm}^3$ and theoretically inferred values for accreting binary systems^{7,1} show a surprisingly small scatter. Can this apparent uniformity of binary pulsars be reconciled with the timing data for (single) radio pulsars, many of which may have also been binaries for some time? In most theories it is assumed that the neutron star is slowed-down by the combined action of a plasma-current torque and a vacuum-wave torque. This has led to the conclusion that there is a deficiency in 'old' pulsars (as measured by their 'slow-down age' $P/\dot{P}$) and that the inferred magnetic moments vary by two orders of magnitude. A worse result is the derived pulsar birth rate² of one per 10 yr if the half life of a pulsar is 10^6 yr as follows from the standard slow-down theory. From these facts we present a new model for pulsar slow-down. The present model predicts four different slow-down epochs from evolutionary changes of the magnetosphere, which are dominated by different braking mechanisms. The model assumes that the masses, magnetic moments and initial rotation periods of all neutron stars are equal. We show that no direct relationship can exist between the 'slow-down age' and the true age of a pulsar and that the pulsar birth rate is one per 100 yr.

Although radio pulsars have been identified with rotating, magnetized neutron stars⁸ and it has been shown that they must be surrounded by a magnetosphere^{9,10} which is independent of the neutron star's surface^{11,12} the progress towards understand-

ing the aspects of the magnetosphere, which determine the slowing-down ('braking') of the neutron star's rotation, has been slow¹³. The theoretical analysis of pulsar braking is hampered by two facts: no self-consistent solution for a pulsar magnetosphere has been found and that the pulsar timing data seem to reveal more about the neutron star's interior¹⁴ than about its magnetosphere. The profusion of radio observations can at best be used as a diagnostic¹³ for the slow-down process. The present model is based on the following assumptions. (1) 'Young' pulsars produce so much plasma by means of 'sparking'^{11,12} that within the plasma no low-frequency wave can propagate^{15,16}. (2) As pulsars 'grow older' sparking becomes less effective so that low-frequency waves can eventually be emitted from within the plasma. (3) The Goldreich-Julian⁹ model as extended to the oblique rotator by Mestel¹⁰ describes the long-term aspects of the magnetosphere out to the velocity-of-light cylinder (that part of the magnetosphere which, were it to corotate rigidly, would rotate at the speed of light). Minor modifications such as a current-regulating net charge and discharges due to radiation friction will be worked into the model later. (4) Considerable counteralignment between magnetic moment and the spin of the neutron star occurs during the first epoch, where the torque is dominated by currents in the plasma (the term 'counteralignment' is used here in the sense of perpendicularity it does not mean magnetic moment and angular momentum becoming antiparallel). The present model accounts for this counteralignment if the star can be treated as a sphere so that free nutation is not possible^{17,18}. However, any other (internal) mechanism which leads to considerable counteralignment will lead to the same consequences (see ref. 18 and refs therein).

The Goldreich-Julian model of the pulsar magnetosphere predicts an excess charge-density in the magnetosphere

$$q = -\frac{\vec{\Omega} \cdot \vec{B}}{2\pi c} \quad (1)$$

and an average current

$$\vec{j} = qc\vec{b}_0 \quad (2)$$

along the open field lines which flow away from the surface area ΔF centred on the magnetic poles (polar caps). Here $\vec{\Omega}$ is the spin angular velocity, $\vec{B}$ the magnetic field, c the velocity of light and $\vec{b}_0$ a unit vector in the direction of $\vec{B}$. For the star not to charge up indefinitely there must be a back-current which flows along magnetic field lines further away from the centre of the polar cap: it will be regulated by a net charge. To close the current, charges must flow within the neutron star across the magnetic field lines and it is this current which breaks the neutron star's rotation. In the Goldreich-Julian model it is assumed that energy and angular momentum are dissipated beyond the velocity-of-light cylinder. Within the velocity-of-light cylinder the charges move along the magnetic field lines like beads on a wire and their current provides a 'magnetic spring' between the neutron star's surface and the matter beyond the velocity-of-light cylinder. Its torque $\vec{T}$ is given by

$$\vec{T} = \frac{1}{4\pi r} \int (\vec{r} \cdot \vec{B}) \vec{r} \times \vec{B} dF \quad (3)$$

where $\vec{r}$ is the radius vector counted from the centre of the star and the integral is taken over a sphere of radius r . The current of equation (2)—in fact any current along the magnetic field lines which brakes the rotation—leads to a counteralignment torque¹⁸ between $\vec{\Omega}$ and $\vec{M}$, in distinction to the torque exerted

by low-frequency waves propagating *in vacuo*, which (if not impeded by nutation¹⁷) leads to alignment^{19,20}. The counteralignment torque can be easily understood by noting that a current flowing through a magnetized sphere will set the sphere into rotation about the magnetic dipole axis $\vec{M}$ and the current of equation (2) is directed (Lenz' rule) so that it reduces the rotation about the original axis.

In the dipole approximation the polar cap surface area ΔF is given by^{9,11} $\Delta F \approx 2\pi R^2 (\Omega R/c)$, where $R \approx 10^6$ cm is the radius of the star and in a coordinate system centred on the magnetic dipole-axis we find for the toroidal component of the magnetic field

$$B_\phi \approx -\frac{\vec{\Omega} \cdot \vec{B}}{2\pi c} \cdot \int \frac{dF}{R \sin \theta} \quad (4)$$

which leads by means of equation (3) to a torque

$$\vec{T}_{pl} = -\alpha \frac{\Omega^2}{c^3} (\vec{\Omega} \cdot \vec{M}) \vec{M} \quad (5)$$

where $\vec{M} = R^3 \vec{B}$ is the magnetic dipole moment and $\alpha \approx 1$. From the corotating part of the magnetosphere we obtain an induced magnetic field parallel to the rotation axis which leads to an extra torque (by means of the magnetic dipole moment $\vec{M}$) on the star

$$T'_{pl} = \frac{\gamma}{Rc^2} (\vec{\Omega} \cdot \vec{M}) \vec{\Omega} \times \vec{M} \quad (6)$$

where $\gamma \approx 1$. Equations (5) and (6) may be compared with the vacuum-wave torque^{17,19,20}

$$\vec{T}_w = -\beta \frac{\Omega^2}{c^3} (\vec{M} \times \vec{\Omega}) \times \vec{M} + \frac{1}{Rc^2} (\vec{M} \cdot \vec{\Omega}) \vec{\Omega} \times \vec{M} \quad (7)$$

with $\beta = 2/3$. The common assumption that both plasma and low-frequency waves will contribute (more or less equally) to the slow-down torque, which leads to the large scatter in inferred magnetic dipole moments will now be shown to be wrong. If low-frequency waves cannot propagate within the plasma they cannot exist outside. Apart from a transition period where the plasma may just allow for low frequency wave propagation (the duration of which is difficult to estimate as it depends on the sparking mechanism) a pulsar is slowed-down therefore exclusively by either the plasma-current torque equation (5) or the vacuum torque equation (7).

The orthogonal rotator provides the most favourable conditions for low-frequency wave-emission and we assume that the plasma which flows out of the velocity-of-light cylinder fills the space about the equatorial plane, consequently the waves are emitted into a plasma dead zone centred on the rotations axis. Let us assume that the two zones are separated by a cone of half-angle ψ (counted from the rotation axis) and as a first approximation¹⁶ that the plasma is infinitely well conducting.

This problem can be solved exactly. Taking the solution of the vector-Helmholtz equation from Morse and Feshbach²³ one finds that the TEM-mode dominates and the dominant radiation mode is given by the lowest n for which

$$\frac{n+1}{n} P_{n-1}^1(\cos \psi) - \frac{n}{n+1} P_{n+1}^1(\cos \psi) = 0 \quad (8)$$

$$P_n^1(\cos \psi) = \frac{n(n+1)}{2} \sin \psi F\left(1-n, 2+n|2\right| \frac{1-\cos \psi}{2}\right) \quad (9)$$

F in equation (9) is the hypergeometric function which for non-integer n is regular in the upper hemisphere where equations (8) and (9) hold. If we let the conducting cone shrink to the equatorial plane one obtains the Deutsch solution²⁴ with $n = 1$. For a thin plasma sheet ($\psi = \pi/2 - \epsilon$) one finds approximately $12 = (1 - \cos \psi) \cdot (n^2(n+2)(n+3) - (1-n^2)(1+n)(2-n))$ (10)

which shows that $n > 1$. The radiated energy rate is

$$\dot{E} = -\frac{2}{2cR^2} (\vec{M} \times \vec{\Omega})^2 \left(\frac{\Omega R}{c}\right)^{2n} \quad (11)$$

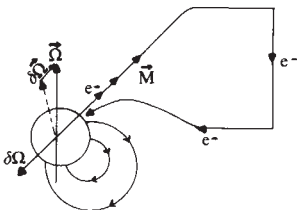


Fig. 1 Schematic current flow which leads to counteralignment. If $\vec{\Omega}$ and $\vec{M}$ are parallel the current flow is metastable. The electron-flow is shown, the algebraic current $\vec{j}$ is directed oppositely. The currents have axial symmetry about the dipole direction $\vec{M}$.

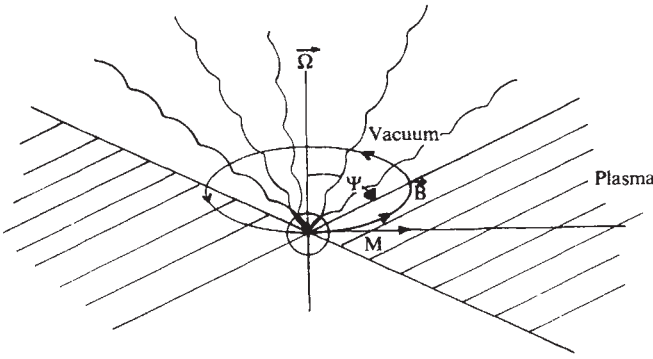


Fig. 2 Plasma-vacuum configuration for which an exact solution of the vector-Helmholtz equation is possible. Hatching indicates the area supposed to be filled with overdense outstreaming plasma in which no low-frequency waves can propagate. Equation (11) gives the energy-loss due to low-frequency waves radiated into vacuum and n is determined from equations (8) or (10).

For a finite thickness of the plasma sheet the emission of low-frequency waves is so strongly reduced ($n = 2$ for $\psi = \pi/4$) that the wave pressure cannot balance the plasma pressure at the boundary and the plasma fills the whole space. Note, however, that if the plasma is asymmetrically distributed in the two hemispheres, such that one cone has $\psi > \pi/2$ (plasma swept backwards, for example, by the pulsar's proper motion²⁵) radiation emission is enhanced and such a state may be called superradiant. A young pulsar will therefore be slowed-down exclusively by the plasma current, an old one by the wave-torque and in the transition epoch we may have

$$\dot{\mathbf{J}} = I \dot{\mathbf{\Omega}} = (\beta - \alpha) \frac{\Omega^2}{c^3} (\tilde{\mathbf{M}} \tilde{\mathbf{\Omega}}) \tilde{\mathbf{M}} - \beta \frac{\Omega^2}{c^3} \mathbf{M}^2 \tilde{\mathbf{\Omega}} + \gamma \frac{(\tilde{\mathbf{\Omega}} \tilde{\mathbf{M}})}{Rc^2} \tilde{\mathbf{\Omega}} \times \tilde{\mathbf{M}} \quad (12)$$

where $\mathbf{J}$ is the angular momentum and I the moment of inertia of the neutron star, $I \approx 10^{45}$ g cm².

Together with the equation of motion for the dipole moment $\tilde{\mathbf{M}}$, which is frozen into the star

$$\dot{\tilde{\mathbf{M}}} = \tilde{\mathbf{\Omega}} \times \tilde{\mathbf{M}} \quad (13)$$

the evolution of the slow-down is easy to determine. Note that equation (12) takes into account only the convection current of equation (2). An additional conduction current due to sparking which may be larger than the convection current will only lead to a different α if the current flows along the magnetic field lines as depicted in Fig. 1. None of the conclusions regarding the first slow-down epoch will be affected as long as $\beta = 0$ as can be seen from equation (14) which becomes independent on α in this case. With the help of the first integral

$$\frac{(\Omega^2 \sin^2 \chi)^\alpha}{(\Omega \cos^2 \chi)^\beta} = \text{constant} \quad (14)$$

where χ is the angle between $\tilde{\mathbf{\Omega}}$ and $\tilde{\mathbf{M}}$ we obtain for young pulsars, for which $\beta = 0$,

$$\sin \chi = \sin \chi_i \left(\frac{\Omega_i}{\Omega} \right) \quad (15)$$

$$\Omega = \Omega_i (1 + ctg^2 \chi_i (1 - e^{-\tau}))^{-1/2} \quad (16)$$

which for small times

$$\Omega = \Omega_i \left(1 - \frac{\tau}{2} ctg^2 \chi_i + \frac{\tau^2}{8} (2ctg^2 \chi_i + 3ctg^4 \chi_i) \right) \quad (17)$$

where $\tau = 2\alpha \Omega_i^2 \sin^2 \chi_i (M^2/Ic^3)t = t/t_e$ is a dimensionless parameter which measures the observer's time t in units of the e-folding time t_e of the model, i refers to initial values.

Before discussing equation (16), we will consider briefly another important parameter for pulsar timing observations. The so-called braking index $N = (\ddot{\Omega}\Omega/\dot{\Omega}^2)$ is given in our model by

$$N = 3 + 2 \frac{(\beta - \alpha)^2 \cos^2 \chi \sin^2 \chi}{(\alpha \cos^2 \chi + \beta \sin^2 \chi)^2} \quad (18)$$

and is never < 3 due to torque minimization. Observationally N is reliably known only for the Crab pulsar^{26,27} where $N \approx 5$. Rewriting the energy balance equation in the form

$$\frac{1}{2} (I \Omega^2)' = -\frac{\alpha}{cR^2} (\tilde{\mathbf{\Omega}} \tilde{\mathbf{M}})^2 \left(\frac{\Delta F}{F} \right)^2 \quad (19)$$

where $F = 4\pi R^2$ is the surface area of the neutron star. A braking index of < 3 may be explained by the pulsar's crust shrinking^{21,22,28} at a rather large rate, or by a slightly larger polar cap $\Delta F/F = (\Omega R/c)^{2/3}$. In fact, some theories²⁹ have the pulse width ΔP and the period P related as $(\Delta P/P)^2 = \Delta F/F$ and the observations of the Crab pulsar, where $\Delta P/P \sim 1/5$ is rather large²⁹, would fit better with $\Delta F/F \approx (\Omega R/c)^{1/2}$ leading to a braking index $N = 3 - 2/3 + 2tg^2 \chi$.

The model is flexible enough to account for the available timing data even under the restriction that all pulsars have the same moment of inertia $I = 10^{45}$ g cm² and the same magnetic moment $M = 10^{30.5}$ G cm³. To determine t_e from the observations we identify those pulsars with anomalously low period derivative with the stars in our model which pass through the end of the first epoch. From the observations $\Omega \approx 2\pi \text{ s}^{-1}$ which gives $\Omega_i \sin \chi_i = 2\pi \text{ s}^{-1}$ so that

$$t_e \approx 10^6 \text{ yr } I_{45} M_{30.5}^{-1} \quad (20)$$

Observationally the two most extreme cases are the Crab pulsar and the binary pulsar. For them equation (20) would lead to $\sin \chi_i = 10^{-1.5}$ and $\cos \chi_i = 10^{-1.5}$ respectively if we assume that both are young objects. To explain the binary pulsar in this way a nearly orthogonal rotator is needed and one may worry whether equation (12) still applies. This case requires $\beta < 10^{-4}$ and $\cos^2 \chi_i < 10^{-3}$. For the binary pulsar $(\Omega R/c)^2 < 10^{-5}$, which guarantees that $\beta < 10^{-4}$ and inspection of the current given by equation (2) shows that it can be closed along the magnetic field lines through the star so that it does not lead to a torque. The braking is then no longer effected by the current of equation (2) but comes about through secondary energy losses such as sparking. Ruderman's estimate¹² of that energy for the (faster) Crab pulsar of 10^{33} – 10^{34} ergs s⁻¹ would lead to the observed braking of the binary pulsar. The first epoch, which lasts some 10^6 yr accounts for roughly a half of the pulsars assuming that all are born as fast rotators. The other half can be explained in the penultimate epoch of pulsar slow-down, where vacuum waves can be emitted in the presence of plasma so that the period derivative goes back to its normal value. Note that in the present model the slow-down age is not related to the true age. The mean active life as determined by Ohmic dissipation can easily exceed 10^7 yr (ref. 18) which brings down the pulsar birth rate by a factor of 10, in comfortable agreement with the more conservative estimates of supernova rates and the lack of discovered neutron stars at their centres³⁶.

We have so far only assumed that the current of equation (2) flows on average without demonstrating how it occurs. Of course, a rigorous demonstration requires a self-consistent solution of the magnetosphere problem, so only the following qualitative argument can be given. According to the Goldreich-Julian model particles cannot stay on open field lines within the velocity of the light cylinder for the same reason that they cannot stay within the star: large electric fields would pull them out. This causes the charge with the correct sign as given by equation (1) to be pulled out, charges with the opposite sign, however, are pulled in on the same field line. Hence a pulsar must have a net charge³⁰ Q to regulate the plasma outflow such that the star does not charge up indefinitely. Some of the charge will be distributed over the polar cap ΔF and most of it over the boundary of the corotating magnetosphere and because it must be able to influence the dynamics of the plasma at the velocity-of-light cylinder it must be of the order of

$$Q = \frac{\tilde{\mathbf{\Omega}} \tilde{\mathbf{M}}}{c} \quad (21)$$

Such a charge reintroduces large electric fields parallel to B , so we have shifted the whole problem from the surface of the neutron star to its velocity-of-light cylinder, sufficiently far away, however, that the star does not get too heated^{1,36}. Note that the net charge as given by equation (21) will not give rise to a back current from the interstellar matter to the pulsar during its active life. This is because the pulsar is well shielded by the electromagnetic fields of the magnetosphere or the vacuum waves which both fall off like r^{-1} whereas the monopole field falls off like r^{-2} so that the force balance is in fact at the velocity-of-light cylinder. It follows that the exact current need not be that given by equation (2) but may be determined by the global magnetospheric structure. As pointed out by Mestel (personal communication) this need not have the symmetry about $\mathbf{M}$, which was crucial for deriving equation (5). In the general case one cannot therefore rigorously predict whether there will be counteralignment or alignment, whereas vacuum waves will always lead to alignment.

In the penultimate slow-down era, which is dominated by low-frequency waves this charge and the corotating (quadrupole) charge of the magnetosphere will also radiate leading to a friction force on the particles in the magnetic field with non-vanishing curl. To compensate for this, the particles must drift across magnetic field lines producing a current which empties the magnetosphere. For the quadrupole radiation from the corotating magnetosphere we get for the time scale of the ensuing discharges some 10^6 pulsar periods and a much shorter time scale for the dipole radiation due to the charge given by equation (21). These discharges may be related to the nulling phenomenon and may give rise to slow-down noise^{27,28} but not to any directly observable speed ups as the inertia involved is too small. The present model does not explain why pulsars turn off unless sparking ceases to be regular enough to allow an observer to detect the object as a pulsar, but even accretion may apparently influence the final era³¹ especially if the pulsar has become an aligned rotator by then.

An attractive explanation is obtained, however, by combining the pulsar extinction hypothesis^{32,33} with the decay of the magnetic dipole moment¹⁸ which regulates the physics at the velocity-of-light cylinder. The observed cutoff period $P \approx 4$ s would then not mainly be a consequence of plasma inertia but rather reflect the time scale for ohmic dissipation in the pulsar's crust which may vary considerably from pulsar to pulsar depending on its thermal history at birth. According to the present model such neutron stars will slow-down in the final era by quadrupole radiation (or plasma currents) on a time scale exceeding the age of the Universe and only if they traverse dense interstellar matter may they be slowed-down more effectively by accretion³¹. The γ -ray transient³⁴ with a period of 8 s could in fact be such an old pulsar, whereas the γ -ray source CG195.5+4.5, if its periodicity of 59.35 s should be confirmed³⁵, should rather not be identified with an old pulsar according to the present model, which predicts ultimate periods around 10 s for dead pulsars, instead of 60 s as deduced by Michel³² assuming that the dipole field does not decay.

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15. Asseo, E., Llobet, X. & Schmidt, G. *Phys. Rev. D* (in the press).
16. Ozernoy, L. M. & Usov, V. V. *Astrophys. Lett.* **13**, 151 (1973).
17. Goldreich, P. *Astrophys. J. Lett.* **160**, L 11 (1970).
18. Flowers, E. & Ruderman, M. A. *Astrophys. J.* **215**, 302 (1977).
19. Davis, L. & Goldstein, M. *Astrophys. J. Lett.* **159**, L 81 (1970).
20. Michel, F. C. & Goldwire, H. C. *Astrophys. Lett.* **5**, 21 (1970).
21. Smoluchowski, R. *Phys. Rev. Lett.* **24**, 923 (1970).
22. Smoluchowski, R. & Welch, D. O. *Phys. Rev. Lett.* **24**, 1191 (1970).
23. Morse, Ph. M. M. & Feshbach, H. *Methods of Theoretical Physics* Vol. 11, 1864 (McGraw-Hill, New York, 1953).
24. Deutsch, A. *Ann. Astrophys.* **18**, 1 (1955).
25. Tademaru, E. & Harrison, E. R. *Nature* **254**, 677 (1975).
26. Groth, E. J. *Astrophys. J. Suppl.* **29**, 453 (1975).
27. Cordes, J. M. *Astrophys. J.* **237**, 216 (1980).
28. Cordes, J. M. & Greenstein, G. *Pulsar Timing IV*, Cornell University Preprint NAIC 138 (1980); *Proc. IAU Symp.* No. 95, 291–298 (1980).
29. ter Haar, D. *Phys. Rep.* **3C**, 59 (1972).
30. Jackson, E. A. *Astrophys. J.* **206**, 831 (1976).
31. Wright, G. A. E. *Nature* **280**, 40 (1979).
32. Michel, F. C. *Astrophys. J. Lett.* **195**, L 69 (1975).
33. Hill, T. W. *Astrophys. Lett.* **21**, 11 (1980).
34. Terrell, J., Evans, W. D., Klebesadel, R. W. & Laros, J. G. *Nature* **285**, 383 (1980).
35. Özel, M. E., Dickel, J. R. & Webber, J. C. *Nature* **285**, 645 (1980).
36. Helfand, D. J. *Nature* **285**, 133 (1980); **283**, 337 (1980).

IR photometry of flat spectrum radio sources

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IR measurements of optically faint, flat spectrum radio sources have implied the possible existence of a new class of quasar, with IR excess more extreme than that of any extragalactic object. In the present preliminary study, 18 Parkes flat spectrum sources have been observed in the IR without regard to optical morphology, and the red sources are found to be just the tail of the normal distribution of quasar colours. The results imply that a considerable fraction of flat spectrum sources from complete samples may have properties similar to the BL Lac objects.

Rieke *et al.*^{1,2} recently detected several objects at $2.2 \mu\text{m}$ in optically blank fields of flat spectrum radio sources^{3,4}, and derived such large IR/optical spectral indices ($\alpha = -d \ln \text{flux density} / d \ln \text{frequency}$) $\alpha_{\text{KB}} \approx 3$ that they proposed a new class of quasar, because other properties of the objects indicated that they were similar to violently variable quasars, or BL Lac objects. They further noted that at the largest (faintest) blue magnitudes, α_{KB} tended to be high, while at $m_B \leq 18$, $\alpha_{\text{KB}} \approx 1$, a value typical for the optically bright quasars of Neugebauer *et al.*⁵. The spectral index α_{KB} was calculated from measurements in the optical B band and the near-IR K band. To test the trend of α with m_B , and to discover whether the red sources represent a new class of quasar, a sample of flat spectrum radio sources was observed in the IR without regard to optical identification. The candidates were selected from the list of 2.7-GHz sources of Condon *et al.*³ according to limits on spectral index ($\alpha < 0.5$, 2.7–5.0 GHz), flux density ($S_{2.7} > 0.5 \text{ Jy}$), RA ($8 \text{ h} < \alpha < 15 \text{ h}$) and dec ($-30^\circ < \delta < -4^\circ$).

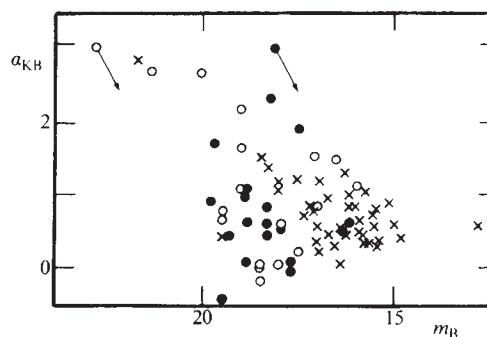


Fig. 1 Optical/IR spectral index versus optical magnitude, showing Rieke *et al.*'s objects^{1,2} (○), the optically bright quasars of Neugebauer *et al.*⁵ (×) and objects from the present study (●). Trajectories show the effect of 1 mag brightening at B between epoch of Palomar plates and IR measurements.

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1. Pines, D. *Science* **207**, 597 (1980); *J. Phys. C2*, 111 (1980).
2. Kundt, W. *Naturwissenschaften* **64**, 493 (1977).
3. Ruderman, M. A. & Sutherland, P. A. *Nature phys. Sci.* **264**, 93 (1973).
4. Levy, E. H. & Rose, W. K. *Nature* **250**, 40 (1974).
5. Taylor, J. H., Fowler, L. A. & McCulloch, P. A. *Nature* **277**, 437 (1979).
6. Trümper, J. *et al. Astrophys. J. Lett.* **219**, L105 (1978).
7. Ghosh, P. & Lamb, F. K. *Astrophys. J.* (in the press); *Proc. IAU Symp.* No. 95, 303–320 (1980).
8. Gold, T. *Nature* **218**, 731 (1968).
9. Goldreich, P. & Julian, W. H. *Astrophys. J.* **157**, 869 (1969).
10. Mestel, L. *Nature phys. Sci.* **233**, 149 (1971).
11. Sturrock, P. *Nature* **227**, 465 (1970); *Astrophys. J.* **164**, 529 (1971).
12. Ruderman, M. A. *Proc. IAU Symp.* No. 95, 87–98 (1980).
13. Mestel, L. *Proc. IAU Symp.* No. 95, 9–24 (1980).
14. Baym, G. & Pethick, Ch. A. *Rev. Astr. Astrophys.* **17**, 415 (1979).

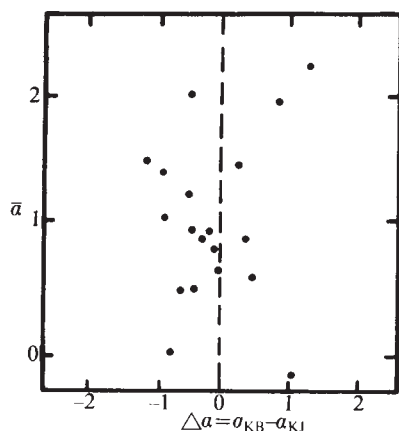


Fig. 2 Plot of mean optical/IR spectral index against the residual $\alpha_{KB} - \alpha_{KJ}$. The deviation from the line $\alpha_{KB} = \alpha_{KJ}$ is a measure of the curvature of the spectrum (either intrinsic or due to variability).

The IR observations were obtained with the Anglo-Australian 3.9-m telescope on 2–3 May 1980 using the InSb Infrared Photometry System. All the observations were made with the broad band K(2.2 μ m) and J(1.25 μ m) filters. Standard stars from the AAO list were observed to calibrate the photometry. The data have been corrected for near-IR extinction at Siding Spring according to recently determined coefficients (G. Gilmore and I. N. Read, personal communication). The data have also been corrected for the effect of different flux distributions on the broad band flux measurements. Only two of the sources lie at galactic latitude $|b| < 30^\circ$, and they were corrected for galactic extinction. The minimum error in the J and K magnitudes was determined from the repeatability of standard observations during a night. In two nights, 21 sources were observed, with 20 detected at K and 18 detected at J.

Table 1 shows the IR data, along with spectral indices calculated between J and K (α_{KJ}) and between the optical B band and K (α_{KB}). There are two major sources of error in these spectral indices; one is the effect of redshifted emission lines passing through the photometric bands. α_{KJ} may be affected, but over the long baseline from 2.2 μ m to the visible, α_{KB} is relatively insensitive to this effect⁶. The second is the fact that the optical magnitude is taken from Palomar plates, and variability might lead to errors in α_{KB} . The photographic fluxes have an uncertainty of ± 0.5 mag (ref. 3).

Figure 1 shows α_{KB} plotted against m_B , with the cluster of red, optically faint objects showing up clearly in the previous data. The 18 sources in our sample present a distribution of spectral indices covering the full range of α from 0 to 3. Very red sources are relatively common; four out of 18 lie in the top part of the

range of spectral indices for BL Lac objects and two are as red as any of those found by Rieke and Lebofsky¹. The data fill in the distribution of very red objects at brighter optical magnitudes, and shows that the trend of α_{KB} with m_B is a selection effect.

Figure 1 shows that much of the argument rests on the α_{KB} indices of the two reddest objects (1335–127 and 1034–293), which may be affected by variability. However, there are reasons to believe that these points are not spurious. First, direct observations on the AAT TV acquisition system confirmed that neither of the objects had varied by more than a magnitude from the epoch of the Palomar plates. The effect of 1 mag variability in B is shown as a trajectory in Fig. 1 both for 1335–127 from the present study and for the reddest object from refs 1, 2. While anomalous brightness at B would bring the two red objects down towards the bulk of the distribution, the same is true of the original red objects. As both sets of α_{KB} were calculated in the same way, the results are directly comparable. Second, there is no evidence from the contemporaneous J and K measurements that these objects are abnormal. Figure 2 shows the mean spectral slope $\bar{\alpha}$ plotted against the difference ($\alpha_{KB} - \alpha_{KJ}$), where the steep spectrum sources lie at the top of the graph. Rieke *et al.*^{1,2} noted that a roughly constant spectral index satisfied both their J, K and optical fluxes; this is represented by the line $\alpha_{KB} = \alpha_{KJ}$ in Fig. 2. Figure 2 also shows that the objects with large α_{KB} do not have abnormally large residuals $\Delta\alpha$, and are not

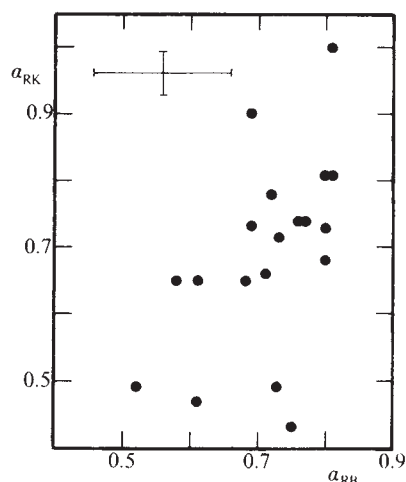


Fig. 3 Plot of ratio/IR and ratio/optical spectral indices. The dispersions in α_{RK} and α_{RH} are a measure of the relative IR and optical flux variations of the sample. Typical error bars are shown in the top left corner.

Table 1 IR data and spectral indices between the J and K, and B and K bands

Source	K(2.2 μ m) (mag)	Flux (mJy)	J(1.25 μ m) (mag)	Flux (mJy)	α_{JK}	α_{KB}	M_B
0823–223	12.47 $\pm$ 0.03	6.79	14.90 $\pm$ 0.03	1.76	2.25	1.5	17.5
0919–260	15.33 $\pm$ 0.07	0.49	16.73 $\pm$ 0.10	0.33	0.68	1.0	19.0
1032–199	15.58 $\pm$ 0.10	0.39	17.16 $\pm$ 0.10	0.22	0.96	0.8	19.0
1034–293	12.48 $\pm$ 0.03	6.69	14.42 $\pm$ 0.03	2.75	1.48	2.4	18.5
1124–186	15.01 $\pm$ 0.04	0.66	17.17 $\pm$ 0.09	0.22	1.85	0.9	18.5
1128–047	15.33 $\pm$ 0.04	0.49	17.13 $\pm$ 0.10	0.23	1.29	1.6	20.0
1143–245	15.14 $\pm$ 0.04	0.58	16.29 $\pm$ 0.05	0.49	0.29	0.8	18.5
1145–071	16.59 $\pm$ 0.12	0.16	17.94 $\pm$ 0.14	0.11	0.68	0.2	18.5
1156–094	15.03 $\pm$ 0.10	0.65	15.42 $\pm$ 0.02	1.09	–0.85	0.3	17.5
1203–261	18.3 (2 σ)	0.03	19.5 (2 σ)	0.02	0.32	–0.5	19.5
1243–072	15.34 $\pm$ 0.07	0.49	17.28 $\pm$ 0.10	0.20	1.51	1.0	19.0
1244–255	14.80 $\pm$ 0.04	0.80	16.32 $\pm$ 0.07	0.48	0.86	0.7	18.0
1256–220	16.42 $\pm$ 0.11	0.18	18.6 (4 σ)	0.05	2.12	0.9	20.0
1302–102	12.88 $\pm$ 0.03	4.71	14.26 $\pm$ 0.03	3.17	0.66	0.6	16.0
1335–127	11.43 $\pm$ 0.03	17.64	13.45 $\pm$ 0.03	6.72	1.61	3.0	18.5
1352–104	15.36 $\pm$ 0.03	0.49	16.77 $\pm$ 0.10	0.31	0.75	0.1	17.5
1354–152	15.23 $\pm$ 0.08	0.54	16.86 $\pm$ 0.08	0.29	1.03	0.7	18.5
1406–076	16.37 $\pm$ 0.11	0.19	18.28 $\pm$ 0.16	0.08	1.46	0.6	19.5
1511–100	>18.8	<0.02	—	—	—	—	18.5
1519–273	17.9 (3 σ)	0.05	—	—	—	—	18.5

segregated from the main body of the distribution. While there is not a 1:1 correspondence between steep α_{KB} and steep α_{KJ} , the four reddest objects in α_{KB} are among the eight reddest objects in α_{KJ} . The means and dispersions of both spectral index distributions are similar ($\bar{\alpha}_{JK} = 1.05 \pm 0.72$, $\bar{\alpha}_{KB} = 0.92 \pm 0.79$). There is no evidence from either the optical or IR data that the red (α_{KB}) objects are other than the tail of the normal distribution of quasar colours. Objects which resemble BL Lac objects in their IR properties are relatively common. The four reddest sources are particularly interesting because (unlike the original 'red' sources) they are bright enough for follow-up spectroscopy and polarimetry. The reddest object (1335–127) has recently been confirmed as a BL Lac object. Our 2.2- μ m measurements with the UK Infrared Telescope show it to have varied by a magnitude over one year, and radio observations by Aller *et al.*⁷ show it to have variable linear polarization at frequencies from 4.8 to 14.5 GHz. In addition, the second reddest object (1034–293) shows evidence of variability ($\Delta m_B > 1$ mag) from UK Schmidt plates of the area, another indication that it is a BL Lac-type object.

Even for this relatively small sample it is possible to draw conclusions on the IR properties of flat spectrum radio sources,

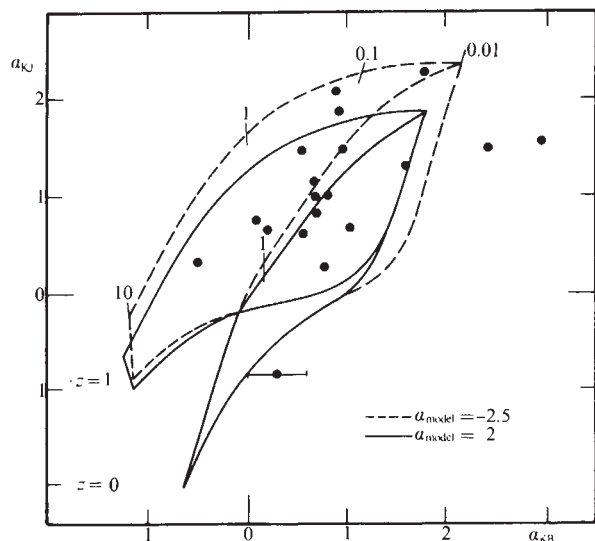


Fig. 4 Plot of near IR and optical/IR spectral indices with permitted zones from a two-component model. The error bars on the lowest point show the effect of a variation of $\pm \frac{1}{2}$ mag in B and typical 5% precision on the J and K flux measurements.

and compare them with the optical properties. Selection effects will not be important because the radio sample was completely identified optically, and with one exception was completely identified at 2.2 μm . Figure 3 shows α_{RK} plotted against α_{RB} , where the spectral index is calculated between the 2.7 GHz measurement and the K and B data points. The range of radio fluxes is less than a factor of 3, while the range of IR fluxes is >500 , thus the graph allows a comparison of the range of IR and optical fluxes in the sample. The mean spectral indices are $\bar{\alpha}_{RK} = 0.69$ and $\bar{\alpha}_{RB} = 0.71$, both very near to the canonical value for optically thin non-thermal radio sources. There is no significant difference between the range of IR and optical fluxes ($\sigma(\alpha_{RK}) = 0.15$, $\sigma(\alpha_{RB}) = 0.08$ and taking the relative standard error in the variance of n variates to be $1.4(n-1)^{-1/2}$) and the even distribution of the very red objects on the α_{RB} axis is a confirmation of the fact that they do not tend to be optically faint. The objects of Rieke *et al.*² do not come from a separate population at better than the 1.5σ level; removing the lower four objects only reduces $\sigma(\alpha_{RK})$ by 0.03 to 0.12.

To see if a simple picture accounts for the range of optical/IR spectra, the energy distribution of a giant elliptical⁸ was added to a power law spectrum of slope α which flattened off ($\alpha = 0$) at some wavelength λ_n (the knee). Figure 4 shows the data plotted on a two-colour diagram of α_{KB}/α_{KJ} . The extreme red objects lie at large values of α_{KB} . As the knee wavelength travels across the spectrum, the source traces a trajectory in the two-colour plane. As the proportion of non-thermal to thermal components is varied, the trajectory sweeps out an area on the diagram. The right-hand boundary represents the extreme non-thermal case of $S_{\text{GAL}}/S_{\text{NT}} = 0.01$, where S_{GAL} is the peak flux of the elliptical galaxy and S_{NT} is the flux at the point where the power law flattens. As the flux contribution of the elliptical increases, the trajectory sweeps across to smaller values of α_{KB} and α_{KJ} , and the left-hand boundary of the enclosed area shows the case where $S_{\text{GAL}}/S_{\text{NT}} = 10$ and the spectrum is dominated by stellar flux. The redshift distribution of flat spectrum radio samples is expected to peak around $z \sim 1$ (ref. 9), and Fig. 4 shows the case for $z = 0$ and $z = 1$. Given the luminosity of a giant elliptical, the flat spectrum sources would be expected to populate the non-thermal ($S_{\text{GAL}}/S_{\text{NT}} < 1$) portion of the $z = 1$ area and the thermal ($S_{\text{GAL}}/S_{\text{NT}} > 1$) part of the $z = 0$ area. Finally the areas have been plotted for two values of α ; $\alpha = 2.0$ and $\alpha = 2.5$ are the solid and dashed curves respectively.

Most of the data points lie well within the area described by the model; the $\alpha = 2.5$ case covering 16 of the 19 sources. Some of the scatter of the data in α_{KJ} will be caused by emission lines

(especially H α passing through the J band) and some of the scatter in α_{KB} may be caused by variability and the non-simultaneity of the optical and IR flux measurements. However, the agreement is surprisingly good given the simple assumptions. A range of α from 2 to 3 is sufficient to cover even the most extreme sources. The assumption that $\alpha = 0$ for $\lambda > \lambda_n$ is not critical. Observations at longer wavelength will help confirm this picture.

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1. Rieke, G. H. & Lebofsky, M. J. *IAU Symp.* **92**, 263–267 (1980).
2. Rieke, G. H., Lebofsky, M. J. & Kinman, T. D. *Astrophys. J. Lett.* **232**, L151–L154 (1979).
3. Condon, J. J., Hicks, P. D. & Jauncey, D. L. *Astr. J.* **82**, 692–700 (1977).
4. Condon, J. J., Jauncey, D. L. & Wright, A. E. *Astr. J.* **83**, 1036–1046 (1978).
5. Neugebauer, G., Oke, J. B., Becklin, E. E. & Matthews, K. *Astrophys. J.* **230**, 79–94 (1979).
6. Hyland, A. R. & Schwartz, M. P. *Proc. astr. Soc. Aust.* **3**, 137–140 (1977).
7. Aller, H. D., Aller, M. F. & Hodge, P. E. *Astr. J.* **86**, 325 (1981).
8. Grasdalen, G. L. *IAU Symp.* **92**, 269–276 (1980).
9. Owen, F. N. & Mufson, S. L. *Astr. J.* **82**, 776–780 (1977).

New family of homogeneous chemical oscillators: chlorite-iodate-substrate

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Interest in oscillating chemical reactions arises because these systems serve as models of temporal organization in biological systems, as starting points for the development of spatial structure in initially homogeneous systems and as instructive examples of the wide variety of dynamic phenomena possible in chemical systems far from equilibrium. One obstacle to the development of a general theory of chemical oscillation has been the small number of fundamentally distinct homogeneous oscillators and the failure of efforts to design new oscillating reactions. We recently suggested a systematic approach to the construction of chemical oscillators and used it to produce a homogeneous oscillating reaction: the chlorite-iodate-arsenite oscillator¹. We now report that the above system is the prototype of a family of homogeneous oscillators, all of whose members have the species chlorite and iodate in common, but which can utilize a wide variety of reducing agents or substrates in place of arsenite.

Figure 1 shows the time dependence of the potentials of an iodide-selective and a platinum redox electrode and of the absorbance at 460 nm when one-electron (ferrocyanide) and two-electron (thiosulphate) reductants react with NaClO₂ and KIO₃ in the same experimental conditions in a continuous flow stirred tank reactor (CSTR). The CSTR maintains the system far from equilibrium, which is a necessary condition for sustained oscillation.

Table 1 gives the concentration range in which oscillations have been found for each of our substrates. Optimum concentrations, at which large amplitude oscillations are observed over a wide range of flow rates (80–2,000 s residence time), are also indicated. While the waveform and frequency of oscillation vary with the flow rate and with the nature and concentration of the substrate, relative changes in $[I^-]$ of 10^4 or more in each cycle were obtained with all substrates. At the upper and lower ends of the substrate concentration range, oscillatory behaviour is observed only at relatively high or low flow rates, respectively. Above or below these concentration limits, only a single non-oscillatory steady state of high or low iodide and iodine concentrations can be observed. The transition from large amplitude

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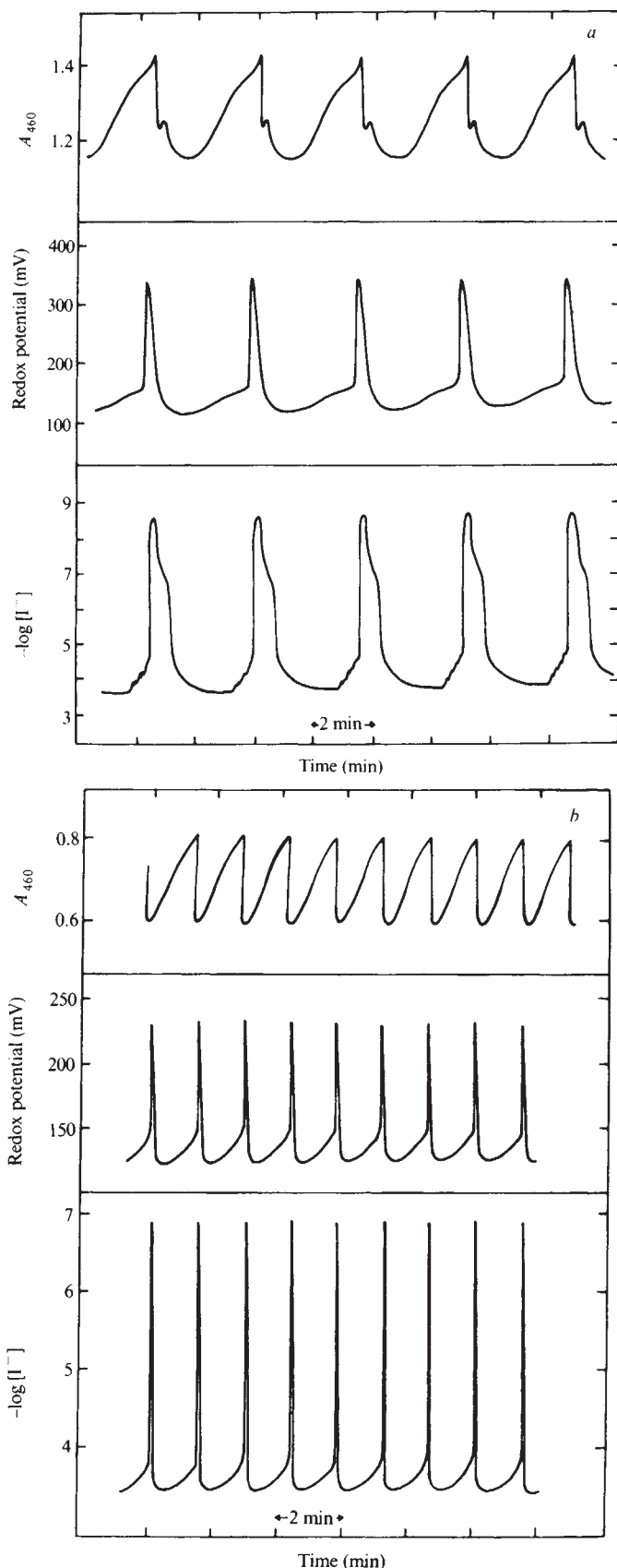
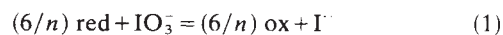


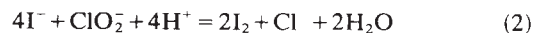
Fig. 1 *a*, Optical density at 460 nm, potential (compared with Hg/Hg₂SO₄ reference electrode) of platinum electrode, and [I⁻] determined by iodide sensitive electrode in a CSTR with input composition (concentrations in the reactor after mixing, but before initiation of reaction), [NaClO₂]₀ = 0.002 M, [KIO₃]₀ = 0.01 M, [K₄(Fe(CN)₆)]₀ = 0.005 M. Temperature, 25 °C; pH, 2.06; residence time, 310 s. *b*, As in *a* but with ferrocyanide substrate replaced by thiosulphate, [Na₂S₂O₃]₀ = 0.005 M, and pH = 2.40.

oscillation to steady state as the substrate concentration is varied may occur either suddenly and directly or through a state of small amplitude oscillation about the steady state which is being approached.

Substrates which generate oscillations include both one- and two-electron reductants and cover a considerable range of redox potentials (see Table 1). Their common feature is apparently that they participate in a Landolt-like reaction².



where red and ox signify the reduced and oxidized form of the substrate, and $n = 1$ or 2 according to whether the substrate is a one or two electron reducing agent. Reaction (1) is autocatalytic in iodide³. The iodide produced then reacts with chlorite to yield iodine:



Reaction (2) is autocatalytic in I₂ and is inhibited by I⁻ (refs 4,5). The chlorite-iodide system gives rise to two different stable steady states in a CSTR⁶. The observed oscillation in the present reaction may then arise from the effect of the relatively slow substrate-generated feedback of iodide on the faster and intrinsically bistable chlorite-iodide subsystem. This view is based on a simple model of the relationship between oscillation and bistability due to Boissonade and De Kepper⁷.

Arsenite reacts less rapidly with iodate than do the other substrates in Table 1. Oscillation with arsenite as substrate must be initiated by the injection of a small amount of iodide into the reactor, while with the other substrates, oscillation begins spontaneously. These observations suggest that initiation of oscillations requires the production of a quantity of iodide above some threshold level.

Neither malonic acid nor iodide as substrate will produce oscillations in our conditions. Malonic acid reacts with iodate to form iodine, which slowly reacts with the excess substrate to yield iodomalonic acid and iodide. The stoichiometry of this process is such that only half as much I⁻ is produced with malonic acid as with the other substrates, and apparently an additional iodide flow is needed to produce oscillation.

The wide range of substrates examined allows us to infer several criteria for a substrate to generate oscillation in this system. The redox potential of the substrate, in the reaction conditions, should be less than that of the iodine-iodide couple (0.62 V (ref. 8)). For example, ferrous ion, hydroquinone and hydrogen peroxide, which react with iodate to yield I₂, have redox potentials of 0.77, 0.70 and 0.68 V respectively, and hence do not give oscillation.

The essential kinetic requirement seems to be an appropriate rate of iodide production either by the direct iodate-substrate reaction or by the subsequent reaction of iodine generated in the initial step with the remaining substrate. If the substrate produces iodide too slowly, then the Dushman reaction ($\text{IO}_3^- + 5\text{I}^- + 6\text{H}^+ \rightarrow 3\text{I}_2 + 3\text{H}_2\text{O}$) prevents iodide from accumulating to the point at which the autocatalytic reaction (2) with chlorite can generate a burst of I₂ to start the oscillation. Thus vanadium (III)

Table 1 Characteristics of substrates which give oscillation with [IO₃]⁻ = 0.01 M, [ClO₂]⁻ = 0.002 M

Substrate	Redox potential* (V)	Concentration range (M)	Optimal concentration (M)	pH
H ₃ AsO ₃ ‡	0.56	0.0024–0.009	0.0075	2.06
Ascorbic acid	0.39†	0.010–0.020	0.010	2.23§
K ₄ (Fe(CN) ₆)	0.35	0.0025–0.010	0.005	2.06
Na ₂ SO ₃	0.17	0.005–0.015	0.010	2.06
Na ₂ S ₂ O ₃	0.08	0.001–0.0125	0.005	2.40
CH ₂ O·HSO ₂ Na	—	0.0025–0.0075	0.005	2.06
Malonic Acid + KI¶	—	0.006–0.16	0.033	2.06

* Ref. 8; † ref. 9.

‡ Iodide initiation (~10⁻⁴ M) is necessary to start oscillation.

§ No oscillation appears at pH = 2.06.

|| Sulphur precipitates at pH = 2.06.

¶ Iodide concentration was kept at 0.0033 M.

has a suitable redox potential ($E^\circ = 0.34$ V), but gives rise only to a stable steady state with low $[I^-]$. Other non-oscillatory substrates which we have tested, such as hydrazine and hydroxylamine, seem to yield iodide too rapidly, thereby inhibiting reaction (2) and forcing the system into a steady state of high iodide concentration. Stannous ion, which seems to satisfy both our redox potential and kinetic criteria, does not give oscillation because the formation of the slightly soluble SnI_2 places a relatively low limit on the iodide concentration that can be attained in the reactor. Substrates which, for kinetic reasons, do not give oscillations in the present conditions, may prove oscillatory in different conditions.

Further studies under way in this laboratory on the detailed mechanism of these systems will hopefully reveal more about the role of the substrate in the family of chlorite-iodate-substrate oscillators.

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1. De Kepper, P., Epstein, I. R. & Kustin, K. *J. Am. chem. Soc.* **103**, 2133–2134 (1981).
2. Bognár, J. & Sárosi, S. *Analyt. chim. Acta* **29**, 406–411 (1963).
3. Eggert, J. & Scharnow, B. Z. *Electrochem.* **27**, 455–470 (1927).
4. Kern, D. M. & Kim, C.-H. *J. Am. chem. Soc.* **87**, 5309–5313 (1965).
5. de Meus, J. & Sigalla, J. *J. chim. Phys.* **63**, 453–459 (1966).
6. Dateo, C., Orbán, M., DeKepper, P. & Epstein, I. R. *J. Am. chem. Soc.* (in the press).
7. Boissonade, J. & De Kepper, P. *J. phys. Chem.* **84**, 501–506 (1980).
8. Latimer, W. M. *Oxidation Potentials* 2nd edn (Prentice Hall, Englewood Cliffs, 1964).
9. Clark, W. M. *Oxidation-Reduction Potential of Organic Systems*, 470 (Williams and Wilkins, Baltimore, 1960).

orthorhombic cell with the above cell dimensions. Averaged intensities for 1,645 unique diffractions with $F_{obs} > 2\sigma$ allowed refinement of the silica framework to $R = 0.10$ with a special least-squares program TWXLLS. The combined intensity of $pI(khl) + (1-p)I(hkl)$ for a pseudo-tetragonal twin was split into the individual intensities $I(hkl)$ and $I(khl)$ for the pseudo-orthorhombic structure where the fractional volume p of one twin component was refined from all the intensities. A difference-Fourier synthesis revealed one organic complex at each of the four intersections of the 10-ring channels (Fig. 1), corresponding to a unit cell composition of 4 TPAF.96 SiO_2 . Least-squares refinement yielded $R = 0.07$ and the atomic coordinates in Table 1. The coordinates of the silica framework are similar to those for silicalite⁹. Because of the restricted data set and some disorder of the TPAF complex, individual isotropic displacement factors were used for the framework atoms and a single displacement factor for the TPAF. No significant electron density was found in a difference-Fourier map based on the coordinates of Table 1.

The silica framework has a mean Si–O bond length of 1.60 Å (σ 0.04) and a range of Si–O–Si angles of 136–178° about a

Table 1 Atomic coordinates for the fluoride silicalite precursor

Atom	x	y	z
Si(1)	0.1213	0.3255	0.9729
Si(2)	0.2741	0.3252	0.9712
Si(3)	0.0768	0.4410	0.8364
Si(4)	0.0712	0.3692	0.1828
Si(5)	0.3144	0.4403	0.8319
Si(6)	0.3080	0.3711	0.1879
Si(7)	0.0733	0.5271	0.1868
Si(8)	0.3080	0.5296	0.1926
Si(9)	0.3127	0.6736	0.8214
Si(10)	0.2799	0.5600	0.9709
Si(11)	0.1227	0.5635	0.9716
Si(12)	0.0762	0.6712	0.8279
O(1)	0.1137	0.2500	0.9287
O(2)	0.1957	0.3464	0.9619
O(3)	0.0825	0.3711	0.8876
O(4)	0.0933	0.3374	0.0843
O(5)	0.2915	0.2500	0.9450
O(6)	0.3155	0.3704	0.8917
O(7)	0.3095	0.3496	0.0732
O(8)	0.9955	0.5423	0.2139
O(9)	0.3702	0.5543	0.2460
O(10)	0.0865	0.5019	0.9174
O(11)	0.0817	0.4514	0.1766
O(12)	0.1150	0.3392	0.2656
O(13)	0.9973	0.3471	0.2054
O(14)	0.3856	0.4432	0.7808
O(15)	0.2564	0.4359	0.7459
O(16)	0.2940	0.4977	0.9096
O(17)	0.3090	0.4481	0.1925
O(18)	0.2547	0.6501	0.7496
O(19)	0.3749	0.3396	0.2391
O(20)	0.0986	0.5612	0.0853
O(21)	0.3070	0.5618	0.0839
O(22)	0.3029	0.7500	0.8487
O(23)	0.3128	0.6299	0.9202
O(24)	0.2036	0.5608	0.9696
O(25)	0.0990	0.6324	0.9269
O(26)	0.0724	0.7500	0.8610
N	0.4478	0.7500	0.1036
C(1)	0.5347	0.7500	0.1373
C(2)	0.5791	0.7500	0.2595
C(3)	0.6115	0.7500	0.3780
C(4)	0.3583	0.7500	0.1430
C(5)	0.3217	0.7500	0.1792
C(6)	0.2586	0.7500	0.1921
C(7)	0.4821	0.6487	0.0649
C(8)	0.5067	0.6454	0.9911
C(9)	0.5023	0.5688	0.9842
F	0.5059	0.7500	0.9366

Standard deviations for Si, O and non-framework atoms are ~0.0006, 0.001 and 0.005 respectively.

Crystal structure of tetrapropylammonium fluoride-silicalite

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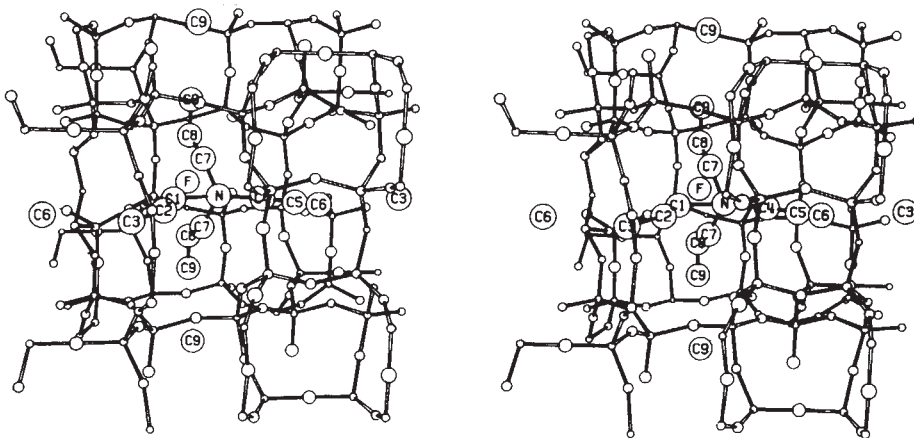
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The hydrophobic and organophilic nature¹ of silicalite may prove commercially important for the removal of organic compounds from waste water. The framework linkage of silicalite¹ is topologically the same as that of synthetic zeolite ZSM-5 (ref. 2), a shape-selective catalyst³ capable of converting methanol into water and hydrocarbons useful in automobile engines⁴. The tetrapropylammonium (TPA) fluoride (F)-containing precursor to fluoride-silicate⁵ crystallizes from a hydrothermal system containing silica, TPA^+ and F^- ions. Destruction of the organic cation during heating in air gives fluoride-silicalite, a polymorph of silica with some properties similar to those of silicalite^{1,5}. We show here how a TPAF complex in the precursor lies at the tetrahedral intersection of the 10-ring channels of a silica framework in a position consistent with a template scheme for crystallization⁶. There is not enough space to permit replacement of TPA by the tetra-*n*-butyl ammonium complex used in the synthesis of ZSM-11 (ref. 7) and its silica counterpart, silicalite-2 (ref. 8).

Crystals of the precursor are interpenetrant twins elongated along the *c*-axis ($180 \times 50 \times 50$ μ m). The twin boundaries are $\{110\}$ and the twin law is 90° rotation about $[001]$. Single-crystal X-ray and optical studies yielded monoclinic symmetry (a , 20.04; b , 19.92; c , 13.39 Å; $\beta \sim 90^\circ$). Because of the near-equivalence of the *a* and *b* axial lengths, the twinning causes near-superposition of *hkl* and *khl* diffractions. The intensities of over 12,000 diffractions, each an *hkl*, *khl* pair, were measured over one quadrant of reciprocal space (MoK α radiation) using a four-circle diffractometer. In spite of the optically monoclinic symmetry, the intensities are consistent with orthorhombic space group *Pnma* used for the refinement of silicalite⁹. The following structure description uses this space group and an

Fig. 1 Stereoview of TPA-F complex and adjacent atoms from the silica framework. ORTEP plot with spheres at 20% probability level.



mean of 153.6° (σ 11.4). A negative correlation between Si–O bond lengths and secant (Si–O–Si) for the 4-coordinated silica polymorphs¹⁰ was interpreted in terms of overlap of molecular orbitals. The present structure shows a similar relationship with slope 0.13 (correlation coefficient 0.6), close to that predicted theoretically for a silica cluster in Fig. 8a of ref. 11.

The tetrahedral configuration of the four propyl limbs of the TPA ion and the close coordination of the fluoride to the central nitrogen at 2.52 Å and four carbons all at 2.21 Å is physically reasonable and resembles that in the crystal structure of TPA-bromide¹². Two propyl limbs partly enwrap the fluorine, and a fifth carbon atom from a third limb lies at 2.75 Å to the fluorine atom. Because hydrogen bonds involving F[−] span a range¹³ of 2.3–2.8 Å which almost encompasses the above distances, the fluoride ion is probably held in place by hydrogen bonding.

The neat fit between the TPAF complex and the internal surface of the silica framework is consistent with a template mechanism for crystallization⁶. Furthermore, the end carbon atom of each propyl limb lies at 2.8–3.1 Å to the end carbon atom of the propyl limb from the next TPAF complex, and there would not be enough space for a butyl limb. Although there are no published atomic coordinates for the structures of ZSM-11 (ref. 7) or its silica counterpart, silicalite-2 (ref. 8) which can be synthesized from tetra-*n*-butylammonium-containing systems, models with plausible geometry show that the channel intersections are larger than for the ZSM-5 and silicalite type of linkage, and in particular are large enough for a butyl-bearing complex. Knowledge of the structural interactions between tetrahedral frameworks and occluded complexes should prove useful in designing conditions for crystallization of useful shape-selective catalysts.

Further data are being collected to increase the precision of the atomic coordinates. The cause of the monoclinic symmetry is also being investigated. The present study has indirect implications for various inorganic–organic complexes, including ones involving clays¹⁴ and biologically important compounds.

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- Flanigen, E. M. *et al.* *Nature* **271**, 512–516 (1978).
- Kokotailo, G. T., Lawton, S. L., Olson, D. H. & Meier, W. M. *Nature* **272**, 437–438 (1978).
- Kokotailo, G. T. & Meier, W. M. *Chem. Soc. spec. Publ.* **33**, 133–139 (1980).
- Meisel, S. L., McCullough, J. P., Lechthaler, C. H. & Weisz, P. B. *Chem. Technol.* **6**, 86 (1976).
- Flanigen, E. M. & Patton, R. L. US Patent NM 4073865 (1978).
- Flanigen, E. M. *Adv. Chem. Ser.* **121**, 119–139 (1973).
- Kokotailo, G. T., Chu, P., Lawton, S. L. & Meier, W. M. *Nature* **275**, 119–120 (1978).
- Bibby, D. M., Milestone, N. B. & Aldridge, L. P. *Nature* **280**, 664–665 (1979).
- Smith, J. V. *Discuss. 5th int. Conf. on Zeolites* (in the press).
- Meagher, E. P., Tossell, J. A. & Gibbs, G. V. *Phys. Chem. Miner.* **4**, 11–21 (1979).
- Newton, M. D. & Gibbs, G. V. *Phys. Chem. Miner.* **6**, 221–246 (1980).
- Zalkin, A. *Acta Crystallogr.* **10**, 557–560 (1957).
- Durrant, P. J. & Durrant, B. *Advanced Inorganic Chemistry* (Wiley, New York, 1964).
- Theng, B. K. G. *Formation and Properties of Clay-Polymer Complexes* (Elsevier, Amsterdam, 1979).

Dehydration-induced luminescence in clay minerals

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Reports of triboluminescent phenomena in organic crystalline materials^{1,2} prompted us to look for related processes in clay minerals. We found the reported extensive mechanical distortion produced on freezing and drying of montmorillonite³ particularly interesting because of our studies of condensation reactions in a wet/dry cycled reaction sequence^{4–7}. We now report the discovery of an unusual luminescent process in several clay minerals and describe its characteristics.

We have found that blue to near UV photons are emitted when a thin layer of an aqueous suspension of certain clays is dried over a desiccant or by gentle heating to 85 °C (ref. 7). Figure 1a–c shows typical examples of three distinctive patterns of photon release as a function of time. The patterns are: (1) a delayed burst, (2) a delayed rise to a slowly decaying plateau and (3) a simple monotonic (exponential-like) decay from the time of introduction of the desiccant.

Kaolinites show an initial monotonically decaying emission of photons followed, some minutes to hours later, by the delayed burst. Two examples are shown in Fig. 1a, Fisher kaolinite and Mesa Alta rock, ground in a mortar and pestle a few days before sample preparation. A similar pattern of emission was observed from all other kaolinites examined; Peerless no. 2; kaolin no. 53 from Birch Pit, Macon, Georgia, and kaolin no. 17 from Lewis-town, Missouri. The photon yield and relative proportion of monotonic to delayed emission varied from kaolinite to kaolinite. The time of onset of the burst is increased by a thicker film. The light is released when the average moisture content approaches 40% H₂O. The significance of this moisture content with respect to water content at the drying front is uncertain, but the average range is within the quoted range for the adsorption and long-range ordering of water by kaolin⁸. Multiple peaks are frequently observed, especially in the non-commercial kaolinites; the peaks seem to be largely, but not fully, explained as experimental artefacts resulting from non-uniformity in the thickness of the clay film and the size of the particles.

The amount of light released on dehydration is an approximately linear function of the amount of kaolin, up to a film thickness of ~10 µm (as calculated from the dry weight of kaolin, the exposed surface area of the emitting film and its estimated density), after which self-absorption and scattering produce a gradual saturation⁷. Light release can be regenerated by repeated cycles of wetting and drying, up to at least four cycles, or until deterioration of the clay film prevents further trials.

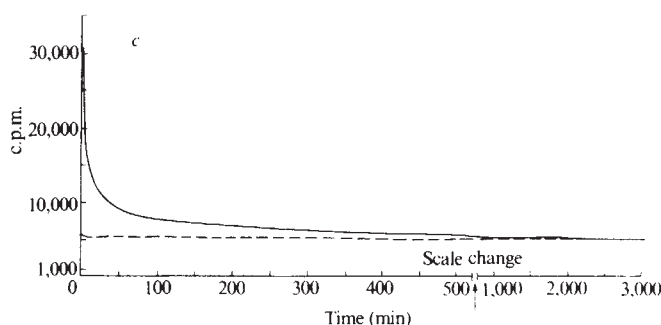
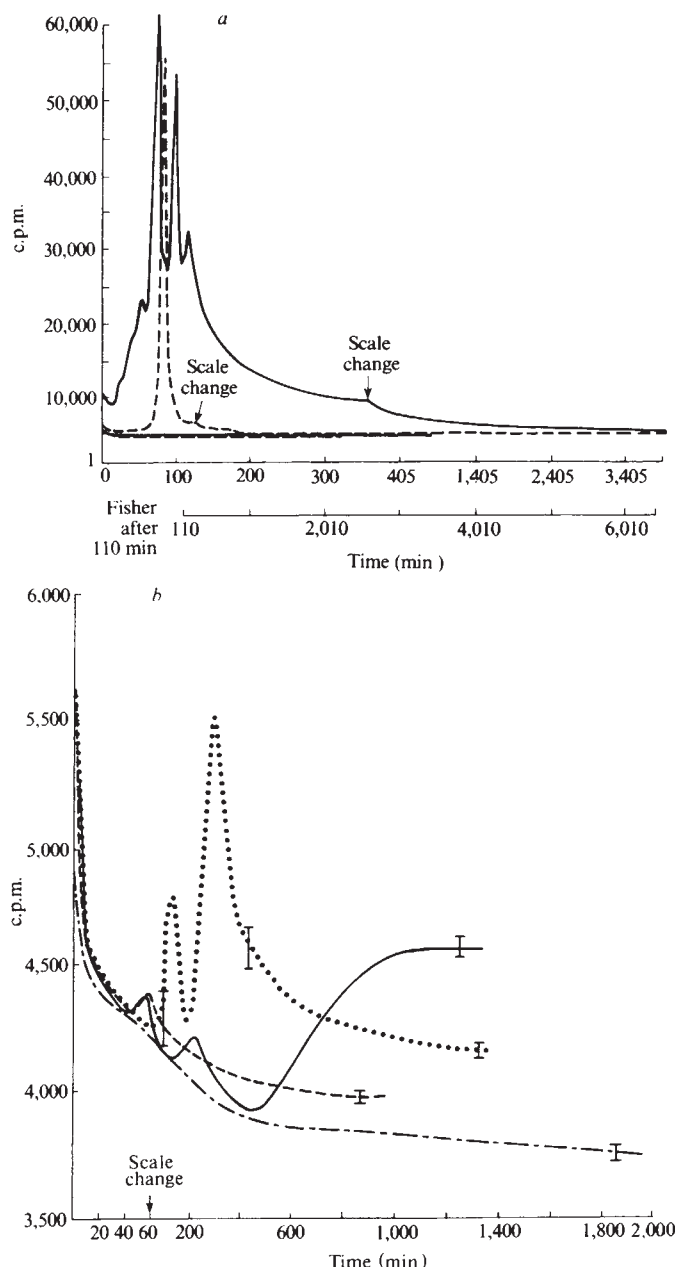


Fig. 1 Dehydration-induced luminescence of several clays. *a*, —, Kaolin no. 9 from Mesa Alta, New Mexico, Wards 48 W 0290 and background (Vial + Drierite) for same—upper timescale (note scale change after 300 min); ---, kaolin, Fisher lot no. 753828 and background (Vial + Drierite) for same—lower time scale after scale change at 110 min. *b*, —, Halloysite no. 13, Dragon Iron Mine, Eureka, Utah; ---, halloysite no. 29, Wagon Wheel Gap, Colorado; ····, halloysite no. 12 48 W 0120, Bedford (Huron), Indiana, North Gardner Mine; — — —, Vial and desiccant background. (The curves for Bedford and Wagon Wheel halloysites have been displaced upwards slightly, for clarity of presentation. The return to background is actually within 100 c.p.m.) *c*, —, Attapulgite of unknown origin; ---, empty tube and tube + drying agent. Luminescence from a clay-water paste (1:1–1:3) was measured as a function of desiccation over CaSO_4 or silica gel. The photon output, integrated over the spectral sensitivity of the EMI 9635 QB Phototube (maximum sensitivity at 3,700 Å), was measured in a Packard 3320 Tri-Carb liquid scintillation counter in the anticoincidence mode with a 50–1,000 window on the discriminators and full gain. Photon output was displayed on a Davidson model 10565 B multichannel analyser. The paste was applied uniformly around the interior of Pyrex scintillation vials, which were scrupulously cleaned in boiling nitric acid and rinsed repeatedly with triply distilled water, air-dried and stored, with their caps, in the dark. Vials were wiped with a damp towel before counting to reduce spurious count-rate from static electricity. Common photon counting artefacts, such as luminescence from the vial, the vial cap or the desiccant, lack of temperature equilibration, and heat release from the wetted desiccant, are negligible after the first 10–30 min, as is clear from the accompanying backgrounds of empty vial and empty vial plus Drierite, and the following three controls. (1) The integrated count rate from the cooling of hot (70 °C) water to the temperature of the counter (10 °C) was 300 counts per g H_2O . (2) The integrated count rate from the addition of water to Drierite was 40 counts per mg H_2O . The clay samples typically contained 30–200 mg of clay and thus 60–400 mg H_2O . Typical peaks had an integrated count of 10^6 counts. (3) No appreciable activity is seen from pastes contained in vials that have been painted black.

Emission from three halloysites is shown in Fig. 1*b*. Bedford shows weak emission and Wagon Wheel shows a pattern similar to that of the kaolinites, but with considerably reduced photon yield. Dragon Iron, however, gives a third characteristic mode of emission: the customary initial monotonic decay is followed by a relatively rapid rise to a slowly diminishing plateau. All three halloysites are colourless, relative to the 2:1-layer clays.

Of several 2:1-layered clays examined, none has shown a delayed peak. For example, montmorillonites, illites and a mica show stronger monotonic components of emission than kaolinites, but no delayed burst. This general form of emission is also characteristic of the attapulgites examined, an example of which is shown in Fig. 1*c*. These clays are more highly coloured than the kaolins; therefore, absence of the delayed burst may be attributable to reabsorption depending on the spectral properties of the different components of the emitted light. However, as the monotonic component is commonly enhanced relative to that in the kaolins, it is reasonable to assume that the differences also imply structurally dependent differences in the modes of emissive relaxation of the materials.

The relative proportion of light emitted as the delayed and as the monotonic components is a sensitive function of a variety of treatments and clay sources. The monotonic component is

increased by orders of magnitude by prior γ -ray irradiation and grinding. In freshly ground samples, it is increased severalfold by a 10-min sonication of the clay/water paste immediately before application. Sonication may simply produce a paste of more uniform particle size by increased dispersion of the larger particles, rather than cause an electronic excitation, but the grinding effect is definitely of triboluminescent origin; this will be reported elsewhere⁹.

An important feature of the delayed peak, with respect to its potential relationship to clay structure–reactivity correlations, is its dependence on the thermal history of the clay. Figure 2 shows the total photon output in the delayed peak after preheating to different temperatures. Heating to temperatures above 500 K temporarily destroys the luminescent effect. Samples of Fisher kaolin preheated to 650 and 1,000 K were remeasured after 6 months, with considerable restoration of the delayed peak in the 650 K sample, but none in the 1,000 K sample. Heating to 650 K for 1 h would not be expected to dehydrate kaolinite irreversibly, but similar heating to 1,000 K would probably result in considerable irreversible dehydration¹⁰. Preheating results in diminution of luminescence induced by both dehydration and grinding. Preliminary results suggest that destruction by preheating, and restoration by γ -ray irradiation, of the

capacity of the material to produce delayed luminescence may resemble similar destruction and restoration of centres active in electron spin resonance which have been identified in natural and doped synthetic kaolins¹¹.

Because of the low overall emissivity of the highly isomorphously substituted 2:1-layer clays and the intermediate emissivity of halloysites relative to kaolinites, we tentatively associate differences in the luminescent yield with those structural parameters that affect the relative magnitude of clay platelet-water and clay platelet-clay platelet attractions such as geometry of platelet stacking or isomorphous substitution. Verification of this hypothesis will rest on detailed study of the relationship between the rate of water loss and photon yield as a function of time in additional specially characterized and purified clays, as clays are notable scavengers, adsorbing and occluding a variety of organic and inorganic materials.

Similar measurements of luminescence have been made on several other naturally occurring, commercially available inorganic compounds such as: TiO_2 , a common contaminant of kaolin; Al_2O_3 and SiO_2 , the chemical constituents of the layered silicates under investigation; hydroxyapatite, a substance with similar high positive-charge deficiency in the crystal lattice; and CaCO_3 , with its characteristic complex thermoluminescent behaviour¹²⁻¹⁴, which we have shown to be more active than kaolin in producing glycine condensation in prebiotic simulation experiments⁷. These materials exhibited no delayed burst and considerable reduction in the monotonically decaying component of light emission. However, calcite, prepared for the measurements by grinding, shows the exponential-like behaviour. Solid material remaining after a 1-week digestion of Fisher kaolin in concentrated HCl showed multiple delayed photon-emission bursts of three-to six fold diminished overall intensity (not shown).

Much work remains in characterizing the spectroscopic properties of the effect—its wavelength dependence, the photon yield, the temperature dependence of the light output, molecu-

lar or atomic details of the trapping site or sites, mechanisms of trap population and energy release, and the relationship between monotonic and delayed emission.

Preliminary studies of the ability of preheated kaolin to produce peptide bonds under the fluctuating reaction protocol indicate an anti correlation between capacity to produce light and capacity to produce peptide bonds; luminescence may well represent wastage of chemically useful energy. Establishment of a relationship between stored electronic energy and the ability of clays to promote chemical reactions would be important to clay chemistry, not only with respect to catalysis in laboratory systems, but also to chemistry in present and past natural environments.

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1. Angelos, R., Zink J. & Hardy, G. *J. chem. Educ.* **56**, 413-414 (1979).
2. Hardy, G. E. *et al. J. Am. chem. Soc.* **99**, 3552-3558 (1977).
3. Cebula, D. J., Thomas, R. K., Middleton, S., Ottewill, R. H. & White, J. W. *Clays Clay Miner.* **27**, 19-52 (1979).
4. Lahav, N., White, D. & Chang, S. *Science* **201**, 67-69 (1978).
5. Lahav, N. & Chang, S. *J. molec. Evol.* **8**, 357-380 (1976).
6. Lawless, J. G. & Levi, N. *J. molec. Evol.* **13**, 281-286 (1979).
7. Coyne, L. M., Lawless, J. G., Lahav, N., Sutton, S. & Sweeney, M. in *Origin of Life*, 115-124 (Reidel, Dordrecht, 1981).
8. Grim, R. E. *Clay Mineralogy*, 251-261 (McGraw-Hill, New York, 1968).
9. Lahav, N., Coyne, L. M. & Lawless, J. G. *Clays Clay Miner.* (in the press).
10. Grimshaw, R. W. *The Chemistry and Physics of Clays* 4th edn (Benn, London, 1971).
11. Angel, B. R., Jones, J. P. E. & Hall, P. E. *Clay Miner.* **10**, 247 (1974).
12. Nishita, H. & Hamilton, M. *Soil Sci* **108**, 1-10 (1969).
13. Ferraresso, G. *Am. Miner.* **52**, 1288-1296 (1967).
14. Aitken, M. J., Fleming, S. J., Reid, J. & Tite, M. S. in *Thermoluminescence of Geological Materials* (ed. McDougall, D. J.) Ch. 3.5 (Academic, New York, 1968).

(100) Deformation twins in naturally deformed amphiboles

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The occurrence of growth twins with (100) as a composition plane has been well established in clinoamphiboles, but no evidence has been presented that (100) twins may form by natural deformation. We now report that in naturally deformed hornblende crystals, selected from an amphibolite mylonite in the Central Scandinavian Caledonides, lamellar (100) deformation twins have been identified using optical and transmission electron microscopy (TEM). The lamellar twins are present within highly bent and kinked parts of the hornblende crystals. Selected area diffraction patterns show that twin and matrix domains are related by a 180° rotation about a^* . Additional defects associated with the (100) planes are unit dislocations with $\vec{b} = [001]$ and stacking faults bounded by partial dislocations. The identification of (100) mechanical twins in clinoamphiboles illustrates the similarity in mechanical twinning behaviour of clinoamphibole and the related clinopyroxene structure.

Deformation experiments on single crystals of clinoamphibole have been described elsewhere¹⁻⁷. The dominant mode of deformation in these experiments is mechanical twinning on (101) in the C2/m setting of the unit cell. Natural occurrences of (101) deformation twins are rare, having only been reported from shock-loaded rocks^{8,9} and in extremely deformed amphibole gabbro from the Ivrea-Verbano Zone in the Alps⁶.

The absence of mechanical (100) twins has not been understood in terms of the crystal structure. In the related clinopyroxene structure two types of mechanical twins occur¹⁰. In experimentally deformed and shock-loaded rocks (001) twins form the analogue with (101) twins in clinoamphibole^{11,12}. The

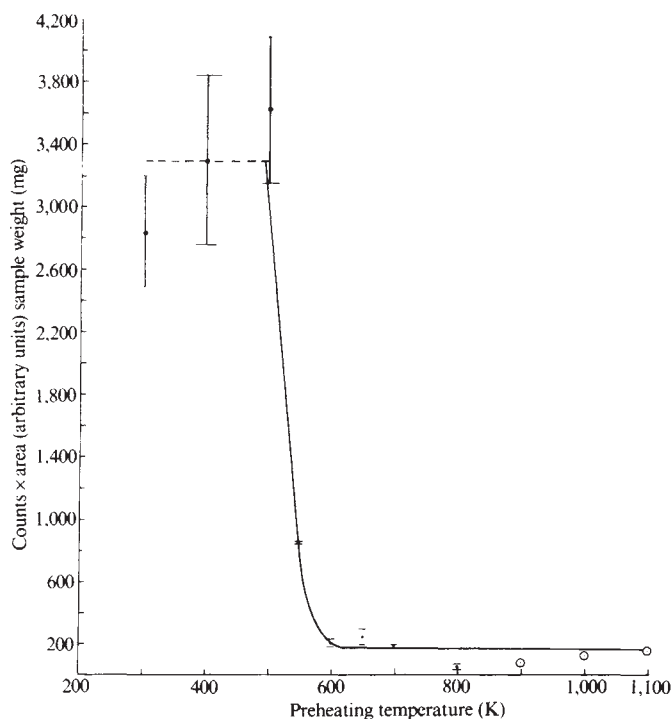


Fig. 2 Effects of preannealing on photon output from kaolin. Samples were heated by placing in a Thermolyne CPS-A8720 oven with a Dubuque III solid-state temperature controller, equilibrated to the nominal temperature, where they were held for ~1 h. Cooling was facilitated by partial opening of the oven door. Samples were removed when the temperature had dropped to below 500 K. The dashed line indicates uncertainty as to whether the apparent rise from room temperature to 500 K is real or an experimental uncertainty.

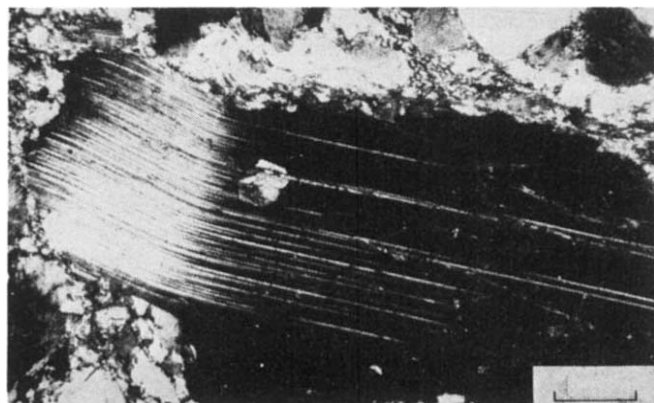


Fig. 1 Bending of a hornblende crystal is accompanied by numerous (100) deformation twins. Amphibolite mylonite Kittelfjäll. Crossed polarized light. Scale bar, 0.1 mm.

(001) plane in clinopyroxene ($C2/c$) is structurally related to the ($\bar{1}01$) plane in clinoamphibole ($C2/m$). In naturally and experimentally deformed clinopyroxene thin lamellar (100) twins are common. There has been no proof that mechanical (100) twins are present in naturally deformed amphibole, although Kirby and Christie¹⁰ have suggested that (100) polysynthetic twins in natural amphiboles may be mechanical in origin. On the other hand, Dolinger and Blacic¹⁴ concluded that (100) [001] slip operates during kinking of hornblendes in experimentally deformed igneous hornblendite and they suggest that (100) [001] slip prevents the occurrence of (100) twinning as it is activated at a lower critical resolved shear stress.

The material described here was selected from a mylonite zone in the basal part of the Kittelfjäll amphibolite massif¹⁵ in the Central Scandinavian Caledonides. The mylonite zone separates the Kittelfjäll amphibolite from the underlying Kittelfjäll peridotite¹⁶. Both lithological units are part of the Seve Nappe, one of the major nappe structures in the Scandinavian Caledonides¹⁷. The mylonite zone formed during the late stages of the Caledonian deformation history at a lower greenschist facies metamorphic regime at intermediate pressures.

Several rock types are present within the mylonite zone, but amphibolites are dominant. In thin section the individual amphiboles are often fractured, bent or kinked, showing numerous lamellar features. The presence of lamellae within the individual crystals is consistently associated with the bent and kinked domains and their number increases towards the more intensely deformed areas (Fig. 1). Based on these observations it was concluded that the lamellae are related to deformation.

Using the optical microscope the crystallographic orientation of the lamellae was shown to be parallel with (100). In sections

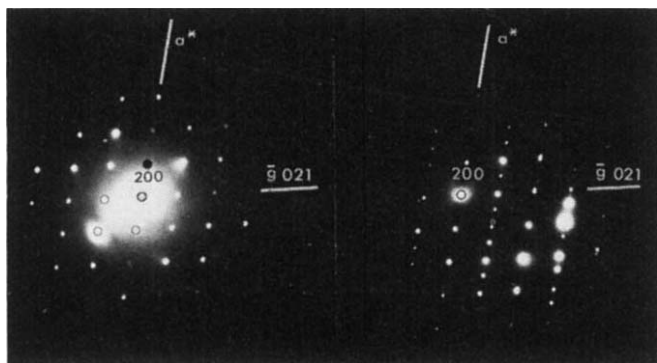


Fig. 2 Selected area diffraction patterns showing the simple pattern of an area from a matrix domain (left) and the complex pattern of an area across the twin interface (right). The extra spots originate from the spots in the left hand pattern by a 180° rotation about a^* . The trace of the twin interface in the corresponding TEM micrograph (not shown) is oriented perpendicular to a^* .

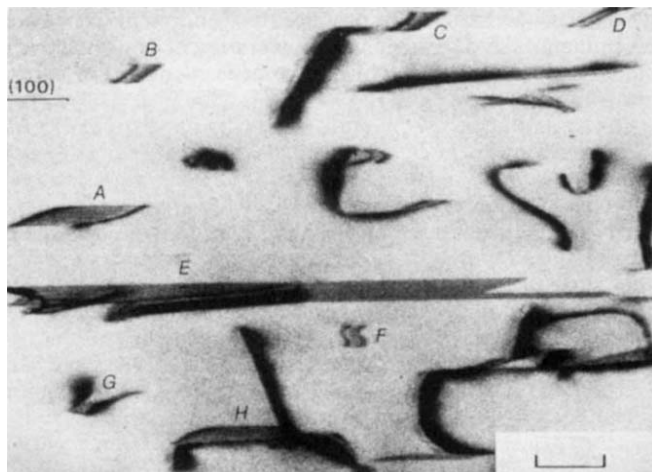


Fig. 3 Partial dislocations at the termination of stacking faults at (A–H). The stacking fault fringes are oriented parallel to the (100) composition planes of the twins. Scale bar, 0.1 μm .

through the crystals parallel to the c -axis the trace of the lamellae extends parallel to the prismatic cleavage. In a few cases the lamellae have also been observed in sections perpendicular to the c -axis. The trace of the lamellae in these sections is parallel to the acute bisector of the dominant $\{110\}$ cleavage planes. When observed in the electron microscope the lamellae appear as long domains, $<20\mu\text{m}$ wide, and bounded by planar coherent interfaces. Chemical analyses on thin foils¹⁸, using an EDAX analytical system, showed no chemical differences between adjacent domains¹³. Selected area diffraction (SAD) showed the twin relation between the adjacent domains. SAD patterns across vertically aligned interfaces produced Laue zones, that contained extra spots, symmetrically distributed with respect to the $h00$ row of reflections (Fig. 2). These extra spots were not present in the electron diffraction patterns from areas selected from a single twin domain.

The $h00$ row of reflections in the complex pattern is common to the diffraction patterns of material at both sides of the interface. The extra spots are related to those of the diffraction pattern from a single twin by a 180° rotation about a^* . This systematic relationship persisted for all diffraction patterns in which the interface was vertical. Dark-field imaging, using twin and matrix reflections, confirmed the above interpretation. In all SAD patterns of areas with vertically aligned twin interfaces the $h00$ row of reflections was perpendicular to the trace of the twin interface in the corresponding TEM images, thus confirming the (100) orientation of the twins.

Additional defect structures observed in the samples are long segments of dislocations parallel with the trace of the (100) planes. Contrast experiments on these dislocations show invisibility for $\bar{g} = 020$ and minimum contrast for $\bar{g} = 200$. It is suggested that the Burgers vector of these dislocations is [001], confirming the slip system identified by Dolinger and Blacic¹⁴ using optical measurements and by Morrison-Smith¹⁹ using TEM. Stacking faults parallel with (100) are present in these samples and are bounded by partial dislocations (Fig. 3).

Mechanical ($\bar{1}01$) twins have not been found in the Kittelfjäll amphibolite mylonites. Mechanical twinning on ($\bar{1}01$) does not in general seem important in the natural deformation of clinoamphiboles. Various authors^{14,19} have demonstrated (100) [001] slip, and the observations on the material described here confirm their conclusions.

The mechanism for the formation of (100) mechanical twins is considered to be the same as that described for clinopyroxene¹⁰. The essential difference between the clinopyroxene and clinoamphibole structure is the presence of double layers of SiO_4 tetrahedrons in the amphiboles. Consequently the b -parameter of the amphibole unit cell is approximately twice that of clinopyroxene, but there are no important differences in the

plane perpendicular to [010]. The geometry of (100) mechanical twinning in clinopyroxene requires the more or less rigid translation of tetrahedral layers by $\frac{1}{2}c$, resulting in an appropriate simple shear deformation of the octahedral layers in between. The composition plane in this model is therefore situated in the tetrahedral layer, which connects the sheared and unsheared octahedral layers. In addition, small shuffles are needed to obtain the exact twin geometry. The twinning shear can be propagated by the movement of partial dislocations with $\vec{b} = \frac{1}{2}[001]$, gliding in the octahedral layer¹⁰. The symmetry operator that is present in the tetrahedral layer of the C2/m clin amphibole structure, is a diad screw axis parallel to b . The twinning mechanism described above will therefore result in a deformation twin with b -glide symmetry on (100).

The study of the deformation behaviour of crystalline silicate materials has become important since interest has concentrated more and more on fundamental mechanisms of deformation. The material from the Kittelfjäll amphibolite mylonites proves that (100) mechanical twinning is a possible mode of deformation in naturally deformed clin amphiboles. The identification of these twins confirms the similarity in twinning behaviour of clin amphiboles and the related clinopyroxenes.

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1. Buck, P. & Paulitsch, P. *Naturwissenschaften* **56**, 460 (1969).
2. Buck, P. *Contr. Miner. Petrol.* **28**, 62–71 (1970).
3. Rooney, T. P. & Riecker, R. E. *Trans. Am. geophys. Un.* **50**, 322 (1969).
4. Rooney, T. P., Riecker, R. E. & Ross, M. *Science* **169**, 173–175 (1970).
5. Rooney, T. P. & Riecker, R. E. *Envir. Res. Pap.* **430**, AFCRL-TR-0045 (1973).
6. Rooney, T. P., Gavasci, A. T. & Riecker, R. E. *Envir. Res. Pap.* **484**, AFCRL-TR-0361 (1974).
7. Rooney, T. P., Riecker, R. E. & Gavasci, A. T. *Geology* **3**, 364–366 (1975).
8. Chao, E. C. T. *Science* **156**, 192–202 (1967).
9. Borg, I. *Am. Geophys. Un. Geophys. Mon.* **16**, 293–311 (1972).
10. Kirby, S. H. & Christie, J. M. *Phys. Chem. Miner.* **1**, 137–163 (1977).
11. Raleigh, C. B. *Science* **150**, 739–741 (1965).
12. Hornemann, U. & Müller, W. F. N. *Jb. Miner. Monatsh.* **6**, 247–256 (1971).
13. Gittos, M. F., Lorimer, G. W. & Champness, P. E. in *Electron Microscopy in Mineralogy* (ed. Wenk, H. R.) 238–247 (Springer, Berlin, 1976).
14. Dolinger, G. & Blacic, J. D. *Earth planet. Sci. Lett.* **26**, 409–416 (1975).
15. Biermann, C. thesis, Univ. Leiden (1979).
16. Calon, T. J. thesis, Univ. Leiden (1979).
17. Williams, P. F. & Zwart, H. J. in *Energetics of Geological Processes* (eds Saxena, S. K. & Bhattacharji, S.) 170–187 (Springer, Berlin, 1977).
18. Lorimer, G. W. & Cliff, G. in *Electron Microscopy in Mineralogy* (ed. Wenk, H. R.) 506–519 (Springer, Berlin, 1976).
19. Morrison-Smith, D. J. *Am. Miner.* **61**, 272–280 (1976).

Increase of CHClF₂ in the Earth's atmosphere

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Ambient air collected and stored in calibration tanks with inert internal surfaces was analysed by the electron capture–gas chromatography techniques (EC–GC) for measuring CHClF₂ (fluorocarbon-22 or F-22) at atmospheric concentrations of between 30 and 60 p.p.t.v. (10^{-12}). Repeated measurements of F-22 in these tanks over the past 18 months have shown no significant changes in concentration, implying that the measurements reflect the atmospheric concentrations of F-22 when the air was collected. The resulting time series, based on 360 measurements made on 100 different samples, shows that CHClF₂ concentrations increased at an average exponential rate of $11.7\% \text{ yr}^{-1}$ (90% confidence limits: $9.8\%–13.2\% \text{ yr}^{-1}$) between April 1976 and January 1981. Based on this time series, it is shown here that a constant $200 \times 10^6 \text{ kg}$ of F-22 in the Earth's atmosphere cannot be accounted for by the global anthropogenic emissions estimated by McCarthy *et al.*^{1,2}. This excess is $\sim 26\%$ of the estimated present global burden of F-22 in the entire atmosphere.

Since the early 1950s, emissions of the inert man-made fluorocarbons CCl₃F (F-11) and CCl₂F₂ (F-12) rose rapidly until 1974, when Molina and Rowland³ proposed that chlorine atoms from the dissociation of these gases could ultimately destroy enough of the Earth's stratospheric ozone layer to affect human health adversely by allowing more UV radiation to reach the Earth's surface. Subsequent theoretical studies also concluded that the large expected accumulations of these gases from continued anthropogenic emissions would lead to a global warming of the Earth's surface, which could cause climatic changes and affect agriculture^{4,5}. In the US these concerns led to restrictions on unessential uses of F-11 and F-12 and a search for substitutes. The National Academy of Sciences (NAS)⁶ suggested that F-22 would be safer for the ozone layer, because it contains only one chlorine atom and some of the F-22 is destroyed in the troposphere by reactions with hydroxyl radicals (HO). The NAS report also cautioned that "... if current trends in the rapidly increasing use of F-22 and methyl chloroform continued unabated, the release rates and atmospheric behaviour of these compounds will require careful attention". F-22 emissions have been increasing rapidly^{1,2}, but so far the total emissions and the atmospheric concentrations remain much less than those of CCl₃F, CCl₂F₂ and CH₃CCl₃. Continuing increases in the emissions of F-22 could, however, lead to a large accumulation in the Earth's atmosphere, partly because it reacts slowly with the hydroxyl radicals, resulting in an atmospheric lifetime of $\sim 20 \text{ yr}$ (ref. 7.). It is believed that reaction with HO radicals is the major global sink of F-22. Furthermore, F-22 efficiently absorbs outgoing IR radiation at wavelengths of $\sim 7.5, 9$ and $12 \mu\text{m}$ (ref. 8) which are, as for CCl₃F and CCl₂F₂, in a region of the spectrum where natural atmospheric gases absorb little of the outgoing radiation. Thus, the general

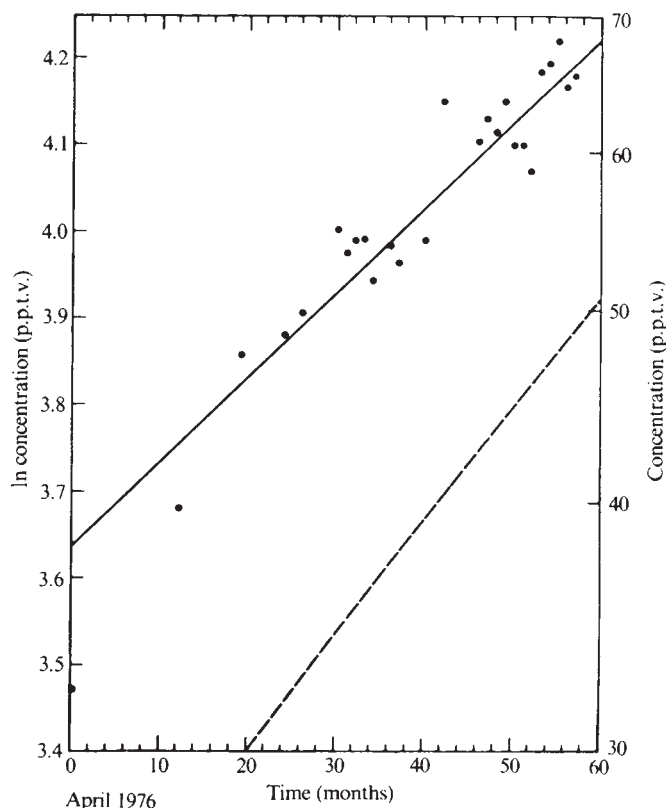


Fig. 1 Monthly average concentrations of CHClF₂ (F-22) over the US Pacific Northwest ($\sim 45^\circ\text{N}$). —, Average concentration line derived by nonparametric statistical methods. ---, Concentrations expected on the basis of global emissions. The error margin for this line is quite large. It is conceivable that when the error estimates are better defined, the two lines will come closer together.

behaviour of F-22 in the Earth's environment is expected to be similar to that of F-11 and F-12, although the magnitudes of possible environmental perturbations may be less and have yet to be determined quantitatively.

The first measurements of F-22 in the atmosphere were made in 1979 by gas chromatography-mass spectrometry (GC-MS) and EC-GC techniques⁹. Ambient air collected periodically since early 1976, before it was possible to measure F-22 at the atmospheric concentration of 30–60 p.p.t.v., had been stored in tanks with inert internal surfaces.

Measurements to obtain a time series were made on about 100 different samples collected between April 1978 and January 1981. An *in-situ* air liquefaction method, which introduces no trace halocarbon contamination, was used to compress about 1,000 l of air at STP to 450 p.s.i.g. and store it in 35 l stainless steel tanks internally passivated by the SUMMA process. The water condensed in the tanks was drained and before analysis the air was further dried to less than 1% RH by a Nafion dryer. The results we report here are F-22 concentrations in dry air.

We believe that the measurements reflect the atmospheric concentrations of F-22 at the time of collection because since we first measured F-22 in the atmosphere more than a year ago⁹, we have made repeated measurements of F-22 in each of our tanks and found no statistically significant systematic changes of concentrations. Random variabilities, however, typically <4% of the initial concentrations, were observed. In addition, repeated measurements over the past 3.5 yr have shown that CCl₃F, CCl₂F₂, CH₃CCl₃ and N₂O have all remained at constant concentrations in all the tanks used for this study, thus establishing the stability of long-lived trace gases in our tanks. These experiments also showed that the precision of analysis was good (estimated $\sigma = 2\%$ and 4% for two tanks studied intensively) whereas the absolute accuracy assigned to the primary standard is only $\pm 10\%$ (estimated σ) (see also ref. 7).

Figure 1 shows the atmospheric concentrations and the increase of F-22 in the atmosphere at 45°N latitude in the US Pacific Northwest. The rate of increase, obtained by non-parametric statistical methods¹⁰, is given in Table 1 and shown in Fig. 1. The statistical techniques used are less sensitive to a few extreme deviations than the classical methods, and the evaluation of the increase by $(1/C)dC/dt = \beta$, where C is the concentration and β the rate of increase, is not sensitive to errors of absolute accuracy.

Next, we estimated the concentrations to be expected from the estimated industrial release of F-22 since 1950⁶.

Table 1 Estimated rates of increase (% yr⁻¹) of CHClF₂ (F-22) in the atmosphere and the estimates of global emissions

	Observed* $\hat{\beta}$ (% yr ⁻¹) (β_L, β_u)		$\beta > 0?$
Measurements (April 1976–January 1981)	11.7 (9.8, 13.2)		Yes ($\alpha < 0.001$)
	Expected † $\hat{\beta}_1$	$\hat{\beta}_2$	
Average (April 1976–January 1981)	11.4	15.9	—
	Global emissions‡ $a(10^6 \text{ kg yr}^{-1})$		r
	b		
January 1950–January 1960	0.12	0.33	0.983
January 1960–January 1968	2.92	0.19	0.998
January 1968–January 1978	16.2	0.153	0.998

* β is assumed constant and given by $(1/C)(dC/dt)$ where C is the concentration. $\hat{\beta}$ is the estimate of β obtained from nonparametric statistical methods based on the Theil test. (β_L, β_u) are the 90% confidence limits of β .

† Expected average rates of increase. $\hat{\beta}_2$ is obtained by a global mass balance and the estimated global emissions. $\hat{\beta}_1$ is the obtained by assuming a constant background (17 p.p.t. at 45°N) in addition to the concentrations expected from anthropogenic emissions.

‡ Functional representation of global emissions based on the model: $S(10^6 \text{ kg yr}^{-1}) = a \exp(bt)$, b in yr⁻¹, t in yr. r is the correlation coefficient of $\ln S$ and t .

Concentrations determined by a global mass balance were corrected for latitude variation⁹ of F-22 and for the amount in the stratosphere⁷ to arrive at the concentrations expected at 45°N latitude¹¹, which are shown in Fig. 1. [The techniques are discussed in ref. 7. It was assumed that the lifetime of F-22 is 20 yr, and that the ratio of C(F-22 at latitudes > 45°N) to C(F-22 at latitudes < 45°S) ≈ 1.3 as determined by measurements made at several times at the south pole, Tasmania and Cape Meares, Oregon, as well as the measurements reported in ref. 9.] We found that the concentration expected on the basis of anthropogenic release estimates is less than that determined by atmospheric observations. We defined the difference as

$$\Delta(t) = C_{\text{obs}}(t) - C_{\text{theor}}(t) \quad (1)$$

The Δ at 45°N was found to be approximately constant with an average value $\bar{\Delta} = 17$ p.p.t.v. (estimated $\sigma = 3$ p.p.t.v., estimated $\sigma/\sqrt{N} = 0.6$ p.p.t.v.). Extrapolating to global scales this amounts to about 200×10^6 kg more F-22 in the Earth's atmosphere than can be accounted for by anthropogenic emissions, and this excess amount has remained nearly constant for the past 4 yr. The independent global F-22 measurements reported earlier⁹ showed the same excess in May 1979. The expected rate of increase since 1976, based on estimates of global emissions, is nearly 16% yr⁻¹, which is faster than the upper limit of the observed rate of increase ($\sim 13\%$ yr⁻¹). This result also suggests that the disagreement between observation and theory is not given by a proportionality constant as would be expected from errors in absolute calibration.

We believe that the most probable explanation for the observed excess is that past industrial releases of F-22 have been underestimated. According to the release estimates of McCarthy *et al.*^{1,2}, only $\sim 41\%$ of the F-22 produced has been released compared with $\sim 85\%$ release/production ratio for CCl₃F and CCl₂F₂, thus allowing for a greater margin of error in the release estimates. If, for instance, we assume that the release to production ratio is $\sim 50\%$ rather than 41%, the discrepancy between our observations and the expected burden disappears. As the published release estimates considered only the production of F-22 used in refrigeration and did not include uses of F-22 as a chemical intermediate for the production of compounds such as polytetrafluoroethylene (PTFE), the amount released may have been underestimated. Estimates of F-22 emissions from eastern Europe, the USSR and China¹¹ are also subject to large uncertainties. Alternative explanations include a possible conversion of CCl₂F₂ to CHClF₂ on sand, particle and other surfaces¹², natural emissions¹³, possible errors in absolute calibration or slow losses of F-22 in the calibration tanks as well as inadequate data on the latitudinal and height distributions of F-22. Work is now in progress to determine the causes of this excess.

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- Jesson, J. P. in *Proc. of the NATO Advanced Study Institute on Atmospheric Ozone* (ed. Aikin, A. C.) 373–396 (US Department of Transportation, Washington DC, 1980).
- McCarthy, R. L., Bower, F. A. & Jesson, J. P. *Atmos. Envir.* **11**, 491–497 (1977).
- Molina, M. J. & Rowland, F. S. *Nature* **249**, 810–812 (1974).
- Ramanathan, V. *Science* **190**, 50–52 (1975).
- Kellogg, W. *Ambio* **9**, 216–221 (1980).
- Stratospheric Ozone Depletion by Halocarbons: Chemistry and Transport* (National Academy of Sciences, Washington DC, 1979).
- Khalil, M. A. K. thesis, Oregon Graduate Center, Beaverton (1979).
- Thompson, B. *Hazardous Gases and Vapors: Infrared Spectra and Physical Constants* (Beckman Instruments, Tech. Rep. No. 595, Fullerton, California, 1974).
- Rasmussen, R. A., Khalil, M. A. K., Penkett, S. A. & Prosser, N. J. D. *Geophys. Res. Lett.* **7**, 809–812 (1980).
- Hollander, M. & Wolfe, D. A. *Nonparametric Statistical Methods* (Wiley, New York, 1973).
- Rasmussen, R. A., Khalil, M. A. K. & Chang, J. S. *Envir. Sci. Technol.* (submitted).
- Ausloos, P., Rebber, R. E. & Glasgow, L. C. *J. Res. natn. Bur. Stand.* **82**, 1 (1977); Pierotti, D., Rasmussen, L. E. & Rasmussen, R. A. *Geophys. Res. Lett.* **5**, 1001–1004 (1978).
- Stoiber, R. E., Leggett, D. C., Jenkins, T. F., Murrman, R. P. & Rose, W. I. *Bull. geol. Soc. Am.* **82**, 2299–2302 (1971).

Cosmogenic ^{10}Be concentrations in Antarctic ice during the past 30,000 years

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We have previously discussed how measurements of the isotope ^{10}Be (half life 1.5 Myr) in geophysical reservoirs can be used to probe variations in the production rate of cosmogenic nuclides^{1,2}. Such variations can be caused by changes in the primary cosmic ray intensity, the geomagnetic field intensity³, and solar activity (through the modulating influence of the solar wind)⁴. We report here the first significant measurements in our programme to determine the ^{10}Be concentration profile over the entire length of a 906-m Antarctic ice core. The results suggest an increased production of ^{10}Be during the Maunder minimum, a period of apparently low solar activity lasting from 1645 to 1715⁵. More surprisingly, we have also found a substantially increased ^{10}Be concentration in snow deposited during the last ice age. While the interpretation of this latter effect is not yet clear, it will almost certainly have important implications for climatology studies. If production variations are indeed involved, there are also important implications for solar-terrestrial relationships and radiocarbon dating.

The measurements were carried out on samples from a 906-m ice core at Dome C (74° 39'S, 124° 10'E) in Antarctica⁶, and from a 180-m core taken a few metres away 1 yr later⁷. We have previously reported some preliminary measurements from the 906-m core, using ^{10}Be extracted from meltwater filters in the field². However, additional studies using laboratory extracted ^{10}Be from ice at the same depths showed that the laboratory extracted experiments often gave higher concentrations⁸. We are not sure whether this is due to adsorption during recovery of the meltwater, or whether the small quantity of ion-exchange resin in the filters became saturated with impurities during the filtering process. All results reported here were made on ^{10}Be extracted from ice in the laboratory. Some results from the upper 250 m of the cores have been reported elsewhere⁹.

Samples consisted of 1–3 kg of ice, each representing at least 10 yr accumulation to minimize possible 11-yr solar cycle effects. The ice was melted in plastic containers, together with 1.5 mg of ^9Be carrier. The Be was then recovered by passing the water through Dowex 50W-8X, 200–300-mesh ion-exchange columns. The Be was eluted with 1.5 M HCl, and then converted to BeO pellets, as described in ref. 1. The technique of measuring the $^{10}\text{Be}/^9\text{Be}$ ratio using the Grenoble cyclotron to determine the ^{10}Be concentration in the ice, has been outlined elsewhere^{2,3} and a more detailed description is given in ref. 10.

The results from 33 samples are shown in Figs 1 and 2. The ages of the samples have been estimated from previous studies on these cores (ref. 11 and M. Pourchet, personal communication). Figure 1 shows results for samples corresponding to the past ~1,000 yr. Our objective was to see whether we could see any medium term (~100 yr) concentration variations, such as those observed for ^{14}C in tree rings¹² which are attributed to solar modulation effects. Although the errors are rather large, we do apparently see an enhanced ^{10}Be concentration about the time of the Maunder minimum (given by Eddy⁵ as lasting from 1645 to 1715). There are not enough points to test whether or not there is evidence for earlier variations such as the Spörer and Wolf minima, and Medieval maximum¹².

Although the experimental uncertainties on the present measurements are quite large, the suggested enhancement during the Maunder minimum (~50%), gives some idea of the potential sensitivity of this procedure for detecting modulation effects. Thus, it is hoped that, not only will it be possible to investigate such variations much further back in time than with ^{14}C tree-ring data, but also that one could establish whether the 11-yr solar cycle itself was operating during such times⁴.

Figure 2 shows results over the whole length (in metres of ice equivalent) of the available cores (for clarity, the 20 points of Fig. 1 have been averaged, and are shown by the open symbol). At depths of more than ~400 m, the ^{10}Be concentration is 2–3 times larger than in the upper part. Figure 2 also shows a smoothed curve of the $^{18}\text{O}/^{16}\text{O}$ ratio (given as $\delta^{18}\text{O}$) measured in the same core¹¹. This ratio is indicative of the temperature at which precipitation occurred, and the large change at ~400 m is believed to correspond to the end of the last ice age, ~10,000 yr ago¹¹. Thus the concentration of ^{10}Be in precipitation in the Antarctic was considerably greater during the last ice age. There are at least three possible explanations for such a situation.

The first, and perhaps the most probable, is that the general rate of precipitation in the Antarctic was much less during the last ice age. Robin¹³, for example, suggests that precipitation in the Antarctic is limited by the saturation vapour pressure of H_2O in the atmosphere. He states that a 7 °C decrease in the average atmospheric temperature, which corresponds approximately to that estimated on the basis of $^{18}\text{O}/^{16}\text{O}$ data¹¹, would be likely to 'halve the accumulation rate of precipitation'¹³. The adopted chronology of Lorius *et al.*¹¹, based on correlation of identified $^{18}\text{O}/^{16}\text{O}$ features with ^{14}C dated sediments and the $^{18}\text{O}/^{16}\text{O}$ variation, assumed a 25% decrease in the precipitation rate beyond ~15,000 yr BP. To account for the ^{10}Be results by this phenomenon, the decrease would have to be considerably greater.

A second explanation related to meteorology is that the circulation pattern in the atmosphere during the ice age led to a greater fraction of the global production of ^{10}Be going into the Antarctic troposphere. This is possible because the largest part of ^{10}Be (and other cosmogenic isotope) production is in the stratosphere¹⁴. How these isotopes reach the Earth's surface depends on the details of stratospheric-tropospheric exchange processes. The features of such transfer which control cosmogenic deposition in Antarctica, are not well understood¹⁵.

The third possibility for the increased ^{10}Be concentration during the last ice age is that the explanation is the same as that for the increase during the Maunder minimum, that is, reduced solar activity, leading to increased ^{10}Be production. If this were the case, one would be tempted to speculate on a causal relationship. The possible connection between reduced solar activity during the Maunder minimum and the 'Little Ice Age' has been discussed extensively^{16–19}.

Fortunately, there are ways of testing which of the above explanations for the increased ^{10}Be concentration is correct. If lower precipitation is the answer, then clearly the ice at the bottom of the Dome C core is considerably older than the present estimate (~50,000 BP instead of ~30,000 BP). Some

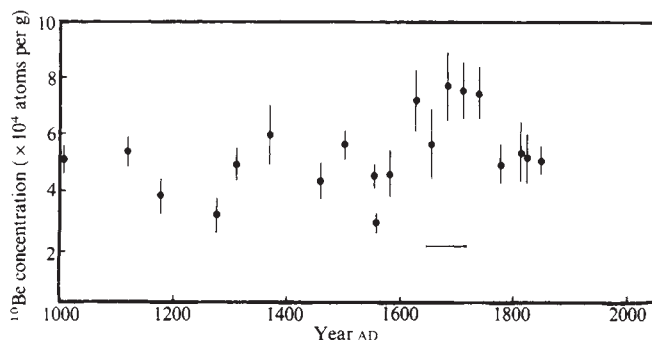


Fig. 1 ^{10}Be concentration in Antarctic ice at Dome C. The solid line corresponds to the Maunder minimum (1645–1715, according to Eddy⁵).

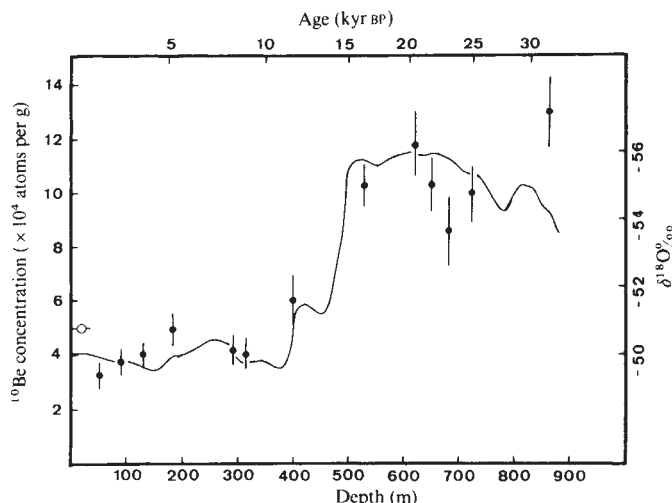


Fig. 2 ^{10}Be concentration (data points, left-hand scale) and $\delta^{18}\text{O}$ (solid line, right-hand scale) in ice at Dome C. The 20 points from Fig. 1 have been averaged, and are given by the open symbol. The $\delta^{18}\text{O}$ data are from ref. 11.

evidence for a longer chronology at Dome C has been presented²⁰. Thus any direct dating of the ice at the bottom of the core would test this hypothesis. The use of the accelerator technique to ^{14}C date the CO_2 trapped in the ice at the bottom of this core might soon be able to provide such a date.

If the explanation is changing atmospheric circulation, then the pre-Holocene deposition of ^{10}Be in other geophysical reservoirs, such as marine and lake sediments, should not all show the same effect (meteorology can change the pattern of ^{10}Be deposition, but cannot increase the total amount deposited). ^{10}Be measurements in these other reservoirs, including an Arctic ice core, are in progress.

If the larger ^{10}Be concentration does reflect increased production before 10,000 BP, then it should be seen worldwide (although not necessarily with the same amplitude). In this case, in addition to the climate implications mentioned earlier, there would be an obvious consequence for ^{14}C dating, as an increased production of this isotope would also be implied. Indeed a combination of ^{10}Be and ^{14}C measurements in the same reservoir could be used to calibrate the ^{14}C scale beyond the presently available tree-ring limit⁸.

Note that we have not considered the possibility that the increase in ^{10}Be could be caused by a decrease in the geomagnetic field intensity. The argument against this is that at ~6,000 yr BP, when palaeomagnetic data suggest a geomagnetic intensity of approximately only half that of the present²¹, the ^{10}Be concentration is still much less than during the earlier period (although there is some hint in Fig. 2 of an increase at ~6,000 yr BP). Thus, while such an effect may be present, it does not seem to be the major cause of the observed increase before 10,000 yr BP. The effect on ^{10}Be deposition of a varying geomagnetic field is probably minimized near the geomagnetic poles⁴.

While many more and precise measurements will be necessary before the implications of the present results can be appreciated, these initial data fully justify our previous enthusiasm^{2,4} regarding the potential of ^{10}Be measurements in polar ice cores.

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1. Raisbeck, G. M., Yiou, F., Fruneau, M. & Loiseaux, J. M. *Science* **202**, 215–217 (1978).
2. Raisbeck, G. M., Yiou, F., Fruneau, M., Lieuvain, M. Loiseaux, J. M. *Nature* **275**, 731–733 (1978).
3. Raisbeck, G. M. *et al. Geophys. Res. Lett.* **6**, 717–719 (1979).

4. Raisbeck, G. M. & Yiou, F. in *Proc. Conf. Ancient Sun* (eds Pepin, R. O., Eddy, J. A. & Merrill, R. B.) 185–190 (Pergamon, Oxford, 1980).
5. Eddy, J. A. *Science* **192**, 1189–1202 (1976).
6. Lorius, C. & Connou, D. *Cour. CNRS* **6**, 17 (1978).
7. Gilet, F. & Rado, C. *Antarctic J. U.S.* **14**, 101 (1979).
8. Raisbeck, G. M. & Yiou, F. *Radiocarbon* **22**, 245–249 (1980).
9. Raisbeck, G. M. & Yiou, F. in *Proc. int. Conf. Sun and Climate* Toulouse (CNES, Toulouse, in the press).
10. Raisbeck, G. M. & Yiou, F. in *Proc. 2nd int. Conf. on Low Level Counting*, High Tatras, Czechoslovakia (in the press).
11. Lorius, C., Merlivat, L., Jouzel, J. & Pourchet, M. *Nature* **280**, 644–648 (1979).
12. Stuiver, M. & Quay, P. D. *Science* **207**, 11–19 (1980).
13. Robin, G. de O. *Phil. Trans. R. Soc. B280*, 143–168 (1977).
14. Lal, D. & Peters, B. *Handb. Phys.* **46**, 551–612 (1967).
15. Maenhaut, W., Zoller, W. H. & Coles, D. G. *J. geophys. Res.* **84**, 3131–3137 (1979).
16. Herman, J. R. & Goldberg, R. A. *Sun, Weather and Climate* (NASA, Washington DC, 1978).
17. Eddy, J. A. *Climate Change* **1**, 173–190 (1977).
18. Robock, A. *Science* **206**, 1402–1404 (1979).
19. Stuiver, M. *Nature* **286**, 868–871 (1980).
20. Duval, P. & Lorius, C. *Earth planet. Sci. Lett.* **48**, 59–64 (1980).
21. Barton, C. E., Merrill, R. T. & Barbetti, M. *Phys. Earth planet. Inter.* **20**, 96–110 (1979).

Molecular carbon isotopic evidence for the origin of geothermal hydrocarbons

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Previous interest in light hydrocarbons from geothermal systems has focused principally on the origin of the methane¹ and the estimation of subsurface temperatures from the carbon isotopic content of coexisting methane and carbon dioxide^{1–3}. Higher molecular weight hydrocarbons were first reported in gases from Yellowstone National Park⁴, and have since been found to occur commonly in geothermal emanations in the western United States⁵. Isotopic measurements of individual geothermal hydrocarbons are now reported which help to explain the origin of these hydrocarbons. The thermal decomposition of sedimentary or groundwater organic matter is a principal source of hydrocarbons in four geothermal areas in western North America.

Steam from production (water-steam) separators at Cerro Prieto (Baja, California, Mexico) or from steam wells at The Geysers (California) was cooled in a stainless-steel condenser. Steam from fumaroles and hot springs at Steamboat Springs (Nevada) and Yellowstone (Wyoming) was sampled with an inverted funnel held underwater over the steam source. All samples were collected in evacuated 500-cm³ Pyrex flasks containing 100 cm³ of 4 M NaOH solution and having Viton O-ring valve seals. Carbon dioxide and hydrogen sulphide readily dissolved in this solution, allowing only the less abundant gases, including the light hydrocarbons, to accumulate in the flask's headspace. This procedure produced negligible amounts of contaminating hydrocarbons. The geothermal hydrocarbons were separated and combusted, and the carbon dioxide product purified and measured using a combined gas chromatograph-combustion system^{6,7}. Hydrocarbon identifications were confirmed by comparing the mass spectra obtained by gas chromatography-mass spectrometry with known standard spectra. The carbon isotopic analyses were performed using a modified Nuclide 6-60 RMS isotope mass spectrometer⁸. The entire procedure was tested using a mixture of hydrocarbons whose individual ^{13}C contents were known. Accurate $\delta^{13}\text{C}$ values were obtained which typically had a standard deviation of 0.3‰.

The gases studied contained most of the possible saturated hydrocarbon isomers ranging in size from methane to the hexanes⁵. Benzene was always relatively abundant, but the

abundances of other unsaturated molecules were relatively low and highly variable between sites. The concentration ranges observed in dry gas for the four typically most abundant hydrocarbons are as follows: methane (25–7,000 parts per million (p.p.m.) by volume), ethane (1–200 p.p.m.), propane (10⁻²–25 p.p.m.) and benzene (10⁻¹ to 15 p.p.m.). The balance of abundant headspace gases included nitrogen, hydrogen, helium and argon.

Concerning the origin of the hydrocarbon mixtures, it is useful to evaluate whether the molecules have attained thermodynamic equilibrium with each other. In an equilibrium mixture at 500 K and 20 atm which has relative carbon, hydrogen and oxygen abundances similar to those in these geothermal fluids, methane should be at least 10⁵ times more abundant than all the larger hydrocarbons⁹. The methane to ethane ratios observed in these samples were always less than 600. The abundances of the other larger hydrocarbons were also greatly in excess of the abundances permitted at equilibrium. Therefore, the molecular patterns of these hydrocarbons reflect, at least in part, the kinetically controlled processes responsible for their production.

Two processes have been proposed for the thermal origin of light hydrocarbons in the Earth's crust. First, methane, which is derived either from storage deep in the Earth¹⁰ or synthesized from carbon dioxide and hydrogen¹, might react with itself to produce larger hydrocarbons^{10,11}. Alternatively, higher molecular weight organic matter from sediments or groundwater might thermally decompose to yield light hydrocarbons^{4,11–13}. The hydrocarbons could then escape thermodynamic equilibration, and hence destruction, by migrating to cooler zones in the Earth's crust. If equilibration is incomplete, the products of

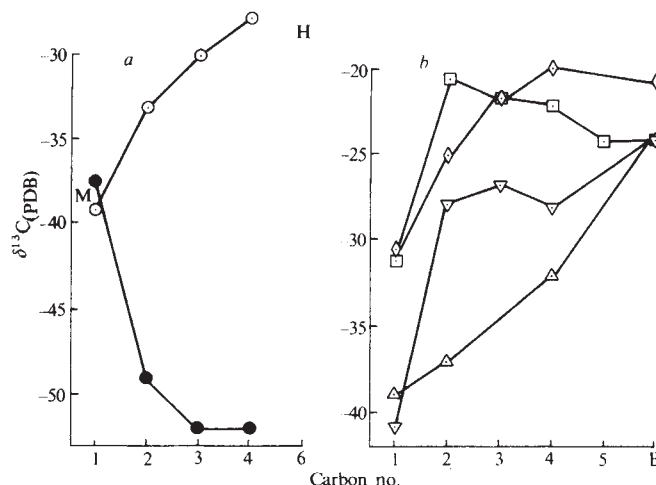


Fig. 2 Plots of $\delta^{13}\text{C}_{\text{PDB}}$ values of individual saturated light hydrocarbons against their carbon number. Numbers on x axis denote carbon number; for example, 1 denotes methane, 2 ethane, and so on. The letter B denotes benzene. *a*, Results of separate laboratory experiments where hydrocarbons were synthesized by thermal decomposition of hexane at 500 °C for 4 h (○) and a spark in a methane atmosphere (●). The procedure for the hexane experiment was the same as that described for Fig. 1*b*. A 2-l Pyrex flask was filled with reagent grade methane ($\delta^{13}\text{C}_{\text{PDB}} = -38.6\%$) to a pressure of 200 mm Hg. The methane was subjected for 1 h to a 2-cm coil spark established between two tungsten electrodes in the flask. The products were extracted from the flask and analysed. The letters H and M identify the $\delta^{13}\text{C}$ values of the hexane and methane, respectively, at the beginning of the pyrolysis and spark discharge experiments. *b*, Isotopic abundance of hydrocarbons from four geothermal localities as follows: □, Cerro Prieto, well M-5, sampled January 1979; ◇, The Geysers, 1979; △, Steamboat Springs, September 1979; ▽, Yellowstone National Park, West Thumb, October 1980.

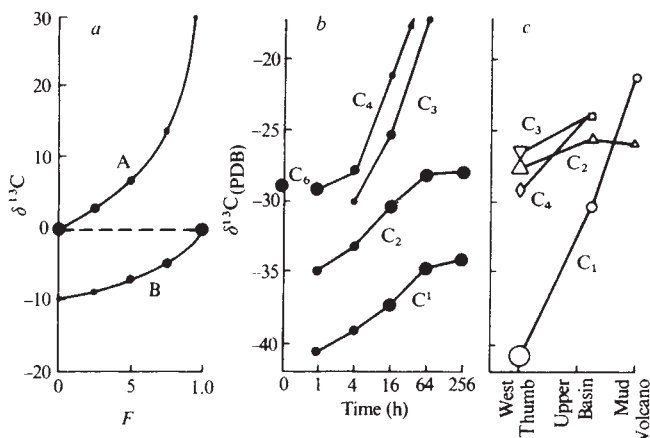


Fig. 1 Relationship between $\delta^{13}\text{C}$ values of hydrocarbon products and certain parameters which control the products' isotopic composition. Sizes of the data points depict qualitatively the relative molar abundances of the individual hydrocarbon species. *a*, $\delta^{13}\text{C}$ values of hypothetical reactant A and product B plotted against F , the fraction of A which has reacted. The curves for A and B have been calculated assuming that the ^{13}C in A reacts at a rate which is 1% lower than that of ^{12}C . The equations used in these calculations were obtained from ref. 14. *b*, Isotopic data from the thermal decomposition of hexane. Approximately 8- μm aliquots of hexane ($\delta^{13}\text{C}_{\text{PDB}} = -29$) were added to 0.4-cm i.d. $\times$ 10-cm long Pyrex tubes with a 10- μl syringe. The tubes were evacuated and glass-sealed while immersed in a -90 °C bath. The tubes were heated at 500 °C for the various times shown and their contents analysed. Notation adjacent to the curves denotes the compounds as follows: C_1 , methane; C_2 , ethane; C_3 , propane; C_4 , butane; C_6 , hexane (the reactant). *c*, The $\delta^{13}\text{C}_{\text{PDB}}$ values of hydrocarbons obtained from three localities in Yellowstone National Park, Wyoming, during October 1980. The $\delta^{13}\text{C}_{\text{PDB}}$ values are plotted against the same y-axis scale used for *b*. Data point symbols denote hydrocarbons as follows: ○, methane (C_1); △, ethane (C_2); ▽, propane (C_3); ◇, butane (C_4); □, mixture of propane and butane. Sizes of symbols depict qualitatively the relative abundances of the hydrocarbons at the three localities. The abundances of hydrocarbons larger than ethane were insufficient at Mud Volcano to be measured isotopically. Both the inferred subsurface temperature and the relative ^3He enrichment in the gases increase (see text), whereas hydrocarbon concentrations decrease, from West Thumb to Upper Basin to Mud Volcano. The $\delta^{13}\text{C}_{\text{PDB}}$ values of CO_2 in the West Thumb, Upper Basin and Mud Volcano samples were -0.2 ± 0.2 , -3.4 ± 0.1 and -2.6 ± 0.2 , respectively. For a given sample, $\delta^{13}\text{C}_{\text{PDB}} = [(\delta^{13}\text{C}/^{12}\text{C})_{\text{sample}}/(\delta^{13}\text{C}/^{12}\text{C})_{\text{PDB}} - 1] \times 1,000$, where PDB is the Pee Dee Belemnite standard.

synthesis from methane should differ in their relative intermolecular ^{13}C abundances from the products of organic decomposition. It is instructive to discuss next the reasons for such a difference.

Consider a kinetically controlled reaction where chemical compound A is converted to compound B and where the isotope ^{12}C in these molecules reacts 1% faster than the ^{13}C . The calculated carbon isotopic compositions of A and B are plotted against F in Fig. 1*a*, where F represents the fraction of A (between zero and unity) which is converted to B. The first B which is produced is almost 10% depleted in ^{13}C , relative to A. As the abundance of A decreases with increasing F , the ^{13}C contents of both A and B must increase to preserve the isotopic mass balance¹⁴. When hexane is thermally decomposed in the laboratory at 500 °C, the ^{13}C contents of the products (see Fig. 1*b*) change in a similar fashion to that depicted in Fig. 1*a*. As with the simple system involving A and B, this increase with time in the ^{13}C contents of the reacting compounds is derived both from isotopic discrimination which accompanies the compounds' production and destruction, and from the requirement that an isotopic mass balance be preserved. Note especially that, in the decomposition process, the lower molecular weight products have lower ^{13}C contents, and that methane has the lowest ^{13}C content of all.

Alternatively, if methane is the carbon source for the thermodynamically unequilibrated synthesis of larger hydrocarbons, these larger hydrocarbons will have lower ^{13}C contents than the methane has. For example, this pattern is observed during the formation of hydrocarbons from methane in a spark discharge (Fig. 2*a*).

The methane in these geothermal samples was consistently more depleted in ^{13}C than were the associated larger hydrocarbons (Figs 1*c*, 2*b*). Thus, the thermal decomposition of organic matter to produce methane and other products is the most important source of these hydrocarbons. This conclusion is supported by our isotopic analyses of coal in drill cuttings from a well in the Cerro Prieto geothermal field. Such coal is a likely carbon source for the light hydrocarbons in the Cerro Prieto steam. The $\delta^{13}\text{C}$ value of the coal (-24.3) is very similar both to the values of the benzene (-24.0) and hexane (-23.6) in the

geothermal steam and to the values of the benzene (-24.6) and butane (-24.9) produced during vacuum pyrolysis of the coal in the laboratory for 16 h at 400°C . Furthermore, methane produced from the coal during this laboratory pyrolysis had a $\delta^{13}\text{C}$ value of -30.0 , very similar to the typical value (-31.4) observed for methane from several Cerro Prieto wells.

If the kinetically controlled geothermal decomposition of sedimentary or groundwater organic compounds produces the gaseous hydrocarbons larger than methane, an appreciable portion of the methane itself must be formed by the same process. If some of this methane is not isotopically equilibrated, as the high abundances of the larger hydrocarbons indicate, then such methane is not suitable for use in a geothermometric method which assumes that carbon isotopic equilibrium has been achieved between coexisting methane and carbon dioxide.

Even though thermodynamic equilibrium is not attained, the carbon isotopic contents of methane and carbon dioxide can give apparent equilibrium isotopic temperatures which are higher in hotter subsurface environments³. An example of this correlation can be found in gases from Yellowstone National Park (Fig. 1c). Maximum subsurface aquifer temperatures are inferred by other methods (ref. 15 and unpublished observations) to decrease from Mud Volcano (steam dominated) to Upper Basin (276°C) to West Thumb (226°C). The $\delta^{13}\text{C}$ values of methane and carbon dioxide (Fig. 1c and its legend) yield calculated isotopic temperatures that are highest at Mud Volcano (428°C), lower at Upper Basin (267°C) and lowest at West Thumb (142°C). The ^{13}C enrichments in methane with increasing temperature could result from kinetic isotope effects similar to those displayed in Fig. 1b. Note in Fig. 1b that the $\delta^{13}\text{C}$ of the methane increased with the extent of the hexane pyrolysis; in that experiment, greater pyrolysis was achieved by longer laboratory pyrolysis times. In the case of the Yellowstone and other gases, a more extensive pyrolysis of sediment or groundwater organic matter could similarly be achieved by higher subsurface temperatures. Consequently, the observed ^{13}C enrichment of methane in the hotter subsurface localities might reflect simply a more extensive pyrolysis of sedimentary organic matter, rather than a thermodynamic equilibration between the carbon dioxide and all the methane which is present.

Alternatively, these Yellowstone gases might contain varying proportions of the methane of deep, unknown origins mixed with methane having a shallower subsurface source. The presence of an abundant unequilibrated shallow methane component is indicated by the occurrence of abundant larger hydrocarbons at West Thumb and Upper Basin. The abundances and ^{13}C contents of these higher hydrocarbons (Fig. 1c) indicate that their associated methane has a relatively low ^{13}C content and has an abundance which decreases, perhaps by a factor of 10 or more, from West Thumb to Upper Basin to Mud Volcano. Accordingly, the total methane content decreases by a factor of approximately eight from West Thumb to Upper Basin to Mud Volcano. Therefore, the relative proportions of ^{13}C -enriched methane having a different and perhaps deeper origin are successively greater at Upper Basin and Mud Volcano. The presence of successively more concentrated deep gas components at Upper Basin and Mud Volcano is consistent with the successively greater enrichments of ^3He observed at these localities (H. Craig, personal communication).

Additional evidence is being sought to establish more definitively the origin of geothermal methane, including that methane which emanates from the ocean floor¹⁶. Nonetheless, it is now apparent that the ubiquitous presence of organic matter in subsurface continental environments has a key role in the production of geothermal hydrocarbons.

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1. Craig, H. *Geochim. cosmochim. Acta* **6**, 53–92 (1953).
2. Hulston, J. R. & McCabe, W. J. *Geochim. cosmochim. Acta* **26**, 399–410 (1962).
3. Panichi, C., Ferrara, G. C. & Confortini, R. *Geothermics* **5**, 81–88 (1977).
4. Gunter, B. D. & Musgrave, B. C. *Geochim. cosmochim. Acta* **35**, 113–118 (1971).

5. Nehring, N. L. & Truesdell, A. H. *Geotherm. Res. Counc. Trans.* **2**, 483–486 (1978).
6. Matthews, D. E. & Hayes, J. M. *Analyt. Chem.* **50**, 1465–1473 (1978).
7. Des Marais, D. J. *Analyt. Chem.* **50**, 1405–1406 (1978).
8. Hayes, J. M., Des Marais, D. J., Peterson, D. W., Schoeller, D. A. & Taylor, S. P. in *Advances in Mass Spectrometry* (ed. Daly, N. R.) 475–480 (Heyden, Philadelphia, 1977).
9. Dayhoff, M. O., Lippincott, E. R., Eck, R. V. & Nagarajan, G. *Thermodynamic Equilibrium in Prebiological Atmospheres of C, H, O, N, P, S and Cl* (NASA SP-3040, Office of Technology Utilization, NASA, Washington DC, 1967).
10. Gold, T. & Soter, S. *Scient. Am.* **242** (6), 130–137 (1980).
11. Galimov, E. M. *Izotopy Ugleroda v Neftegazovoy Geologii* (Nedra, Moscow, 1973).
12. Craig, H. in *Nuclear Geology of Geothermal Areas* (ed. Tongiorgi, E.) 17–54 (CNR Laboratorio Di Geologia Nucleare, Pisa, 1963).
13. Silverman, S. R. *Proc. 8th Wild Petrol. Congr.* **2**, 47–54 (1971).
14. Melander, L. & Saunders, W. M. Jr *Reaction Rates of Isotopic Molecules* (Wiley, New York, 1980).
15. Truesdell, A. H. & Fournier, R. O. *Conditions in the Deeper Parts of the Hot Spring Systems of Yellowstone National Park, Wyoming* (Open File Rep. 76–428, US Geological Survey, Menlo Park, 1976).
16. Welhan, J. *EOS* **61** (46), 0–120 (1980).

Lower Palaeozoic strata on the Pacific Plate of North America

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The portion of California (USA) and Baja California (Mexico) lying west of the San Andreas–Gulf of California plate boundary is part of the Pacific Plate, and consists of at least seven distinct lithological terranes (Fig. 1). The only pre-Carboniferous rocks previously identified in any of these terranes are the Precambrian gneiss and plutonic rocks of the San Gabriel Mountains in California (Fig. 1, terrane III). Previous reports of Palaeozoic strata west of the San Andreas Fault in southern California are either erroneous¹ or unconfirmed (R. V. Sharp, personal communication). Fossils near El Volcan, Baja California, originally reported as possibly Palaeozoic², are now recognized to be early Triassic³. Unidentified cup corals and crinoid-like specimens were reported in metamorphosed limestone of the Gabilan Range, central California (Fig. 1, terrane I)⁴. Unidentified brachiopods and crinoidal debris in the Sierra Pinta of Baja California were assigned to the Carboniferous (Fig. 1, terrane VII; ref. 5). Fossils of Pennsylvanian age were found in a block of limestone and quartzite in a *mélange* on Isla Cedros.⁶ We describe here the first Lower Palaeozoic rocks from the Pacific Plate; this carbonate-quartzite sequence contains Lower Ordovician conodonts and is exposed at San Marcos, 50-km south of the International Border between Tecate and Ensenada (Fig. 1).

The San Marcos section consists of carbonate, quartzite, bedded chert and subordinate slate, and is exposed in a tectonic envelope ~6 km long and 1 km wide. Preliminary mapping suggests that the rocks were thrust over sandstone-slate turbidite deposits presently considered to be of Triassic or Jurassic age (Fig. 1, terrane V). Following tectonic emplacement both the upper and lower plates were folded, weakly metamorphosed and intruded by Cretaceous granite and rhyolite/andesite dykes.

Table 1 Conodont taxa from sample VS3

Multi-element taxa		
<i>Drepanodus arcuatus</i> Pander	NA	vc
<i>Eoplacognathus</i> ? sp.	NA	vr
<i>Prioniodus</i> (<i>Oepikodus</i>) <i>evae</i> (Lindström)	NA	vc
<i>Prioniodus</i> (<i>Periodon</i>) <i>flabellum</i> (Lindström)	NA	c
<i>Protopanderodus varicosatus</i> Sweet & Bergström	NA	vc
<i>Scolopodus cornutiformis</i> Branson & Mehl	M	c
<i>Walliserodus ethingonti</i> ? (Fähræus)	NA	r
Form taxa		
<i>Cordylodus</i> ? sp.	M?	vr
Oistodiform element	?	c
Scandodiform element	?	r
Trichonodelliform element	?	r

NA, North Atlantic elements; M, mid-continent elements; vr, very rare; r, rare; c, common; vc, very common.

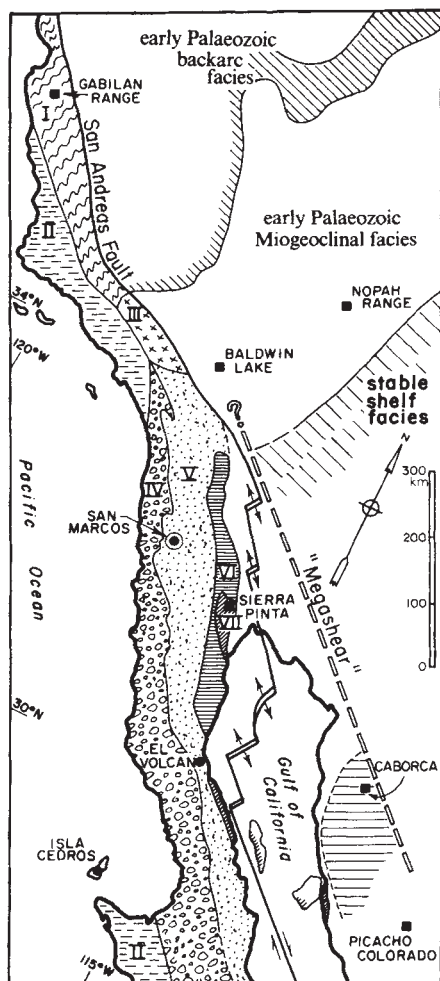


Fig. 1 Index map of seven pre-batholithic basement terranes of the Pacific Plate of western North America. I, Salinian block of highly metamorphosed carbonate-argillite of pre-Cretaceous age. II, Ophiolite, *mélange* and associated trench facies; Triassic to Cretaceous in age. III, Igneous rocks and gneiss of Precambrian age. IV, Island arc rocks of late Triassic to medial Cretaceous age. V, Flysch facies of early Triassic to late Jurassic age. VI, Amphibolite grade carbonate-quartzite rocks of unknown age. VII, Chert-argillite-volcanic rocks of Carboniferous age. Structural features, geographic locations and boundaries of shelf, miogeoclinal and back-arc facies are indicated. Conodont-bearing rocks occur at San Marcos within terrane V, latitude 116°25'33"W, longitude 32°09'46"N. Samples were collected on a saddle of a north-west trending ridge at an elevation of ~750 metres, Hoja 111D82, Francisco Zarco quadrangle, Baja California, Mexico.

Lithological specimens ranging from reddish-brown weathered, dark-grey, fine- to medium-grained dolomitic limestone to very dark-grey, fine-grained dolomitic limestone were collected from limited exposures on brush-covered slopes. Six samples, weighing 535–1,025 g, were dissolved in 10–12% acetic acid. Fossils were recovered from three samples, but only one (VS3, 797 g) contained abundant conodonts (~250) and other fossils, including inarticulate brachiopod fragments, small gastropod steinkerns, pelmatozoan columnals and sponge? spicules. Only the conodonts are preserved well enough for more specific identification.

Most of the conodonts are broken, etched, compressed and have been thermally altered to a dark brown or black (CAI index of 5–6)⁷. Despite poor preservation seven multi-element taxa and four other morphological types were identified (Table 1, Fig. 2). With the exception of fragments tentatively identified as *Eoplacognathus?* sp., other species are characteristic of the *Prioniodus evae/Oepikodus evae* Biozone of early Ordovician (medial Arenigian) age as recognized in the British Isles^{8,9}. These taxa and *Scolopodus cornutiformis* suggest a correlation with early Ordovician Faunas D/E of late Canadian age as described for the US mid-continent¹⁰. Therefore the conodonts provide evidence of close correlation with European biozones and series, but provide only tentative correlation with North American mid-continent taxa.

These taxa are representative of two Ordovician biogeographic provinces. Six species, which represent most of the recovered specimens, are diagnostic of the North Atlantic province^{11,12}; two species are diagnostic of the North American mid-continent province (Table 1)^{11,12}. The diversity and abundance of the specimens indicate that these rocks formed in a North Atlantic province setting. In North America, rocks containing medial Arenigian conodonts of this province have been reported from the northern Appalachians, the Marathon region in Texas, and from the Cordilleran region of Canada and the United States¹². Medial Arenigian rocks outside North America that contain conodonts of this province have been reported from central, western and southern Europe, south-east Asia, Australia and Argentina^{9,11,12}. The distribution of these localities on Ordovician palaeogeographic maps¹³ indicates that all occur at least 10–15° north or south of the Ordovician palaeoequator. Thus the conodont taxa, the abundance of specimens in the dissolved sample, and the lithology indicate the rocks were deposited on a continental shelf (miogeocline) in subtropical to temperate palaeoclimates.

These Lower Palaeozoic miogeoclinal rocks are isolated from other known outcrops of carbonate rocks, and lithological relationships with other sections are very tentative. The San Marcos exposures are 70 km south-west of strongly metamorphosed quartzite-carbonate rocks of unknown age found in the desert

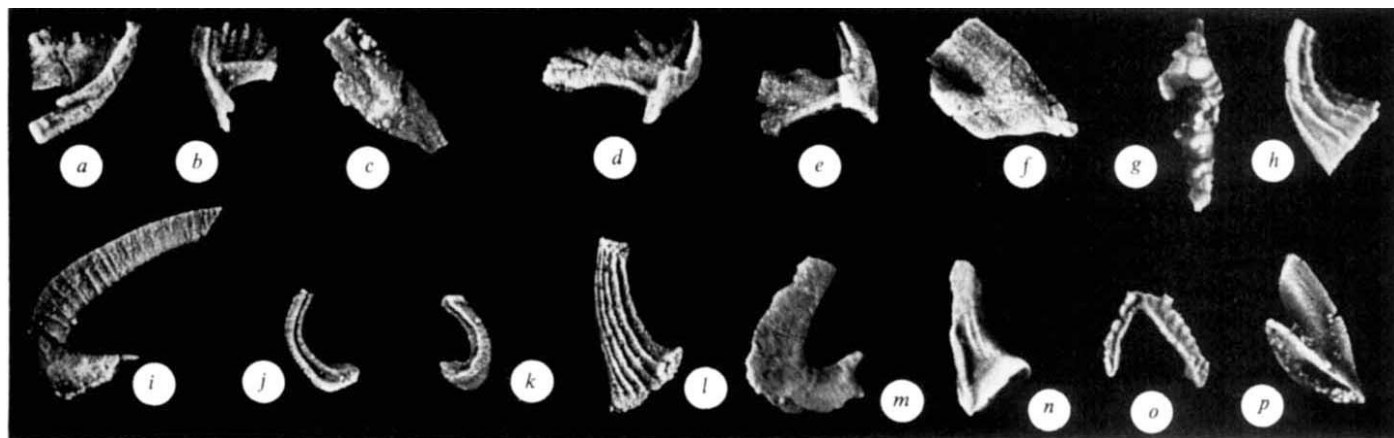


Fig. 2 Conodonts from sample VS3. All specimens are from SDSU location number 3236, Allison Center, Department of Geological Sciences. Specimens i, j, k, l, m, and p are magnified by ~22 diameters, all others by ~35 diameters. a, b, c, *Prioniodus (Oepikodus) evae* (Lindström), oepikodiform, prioniodiform and oistodiform elements; d, e, f, *Prioniodus (Periodon) flabellum* (Lindström), ramiform, ramiform and oistodiform elements; g, *Eoplacognathus?* sp.; h, *Walliserodus ethingtoni?* (Fähræus); i, *Drepanodus arcuatus* Pander; j, k, *Protopanderodus varicosatus* Sweet & Bergström; l, *Scolopodus cornutiformis* Branson & Mehl; m, *Cordylodus?* sp.; n, scandodiform element; o, trichonodelliform element; p, oistodiform element.

ranges west of the plate boundary (Fig. 1, terrane VI). On the east side of the plate boundary, in northwestern Sonora near Caborca (Fig. 1), is a relatively unmetamorphosed sequence of miogeoclinal rocks of late Precambrian and Cambrian ages^{14,15} that was correlated with the miogeoclinal succession of the Nopah Range in the southern Great Basin of California¹⁶. No Ordovician rocks were recognized in the Caborca section, but Upper Ordovician strata of deep water facies occur 200 km to the south at Picacho Colorado (Fig. 1)^{17,18}. Another possibly related section of carbonate-quartzite rocks, near Baldwin Lake in the San Bernardino Mountains, California (Fig. 1), was also correlated with Cambrian and older rocks in the Nopah Range^{19,20}.

Despite difficulties in establishing lithological relationships, this well documented section of Ordovician miogeoclinal rocks west of the plate boundary is important in formulating interpretations of Palaeozoic and younger tectonic history of the southwestern part of the Cordilleran geocline. Geologists have long speculated about the possible truncation and/or displacement of geocline rocks in areas that now represent the Mojave desert, Baja California, and northern Sonora. Anderson and Silver²¹ suggested that some of these rocks, such as those represented by the Caborca sequence, were transported south-eastwards some 700 km along a left-lateral 'megashift', perhaps in medial Jurassic time. Others²² have suggested that displacement of the miogeoclinal rocks was to the north-west. Whether or not the Caborca rocks were once adjacent to the southern Great Basin/Mojave Desert, the carbonate-quartzite terrane of the desert ranges (Fig. 1, terrane VI) is now separated from the western edge of the Caborca terrane by more than 300 km across the northern Gulf of California rhombochasm. The sense of motion and distance agree with independent evidence for plate boundary separation across the Gulf^{23,24}. Palaeomagnetic studies, however, suggest that northern Baja California was much further south relative to the rest of North America at the time of Cretaceous granitic intrusion^{25,26}. Resolution of these palaeogeographic arguments is not yet possible. The Lower Ordovician carbonate-quartzite rocks of San Marcos, Baja California must be taken into account in reconstruction of displaced terranes along the western margin of North America during the past 100 Myr.

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- Schwartz, H. P. *Geol. Soc. Am. Spec. Pap.* **100** (1969).
- Gastil, R. G. *et al. Geol. Soc. Am. Mem.* **140** (1975).
- Gastil, R. G. *et al. Geol. Soc. Am. Abstr. Prog.* **13**, 57 (1981).
- Bowen, D. E. & Gray, C. H. *Jr California Division of Mines, Spec. Rep.* **56** (1959).
- McEldowny, R. C. thesis, San Diego State Univ. (1970).
- Kilmer, F. H. in *Baja California Geology* (eds Abbott, P. L. & Gastil, R. G.) 11–28 (San Diego State University, 1979).
- Epstein, A. G. *et al. U.S. geol. Surv. Prof. Pap.* **995** (1977).
- Lindström, M. *Geol. Soc. Am. Mem.* **127**, 21–61 (1977).
- Löfgren, A. *Fossils and Strata* **13**, 1–129 (1978).
- Ethington, R. L. & Clark, D. L. *Geol. Soc. Am. Mem.* **127**, 63–82 (1971).
- Bergström, S. M. in *Atlas of Paleobiogeography* (ed. Hallam, A.) 47–58 (Elsevier, Amsterdam, 1973).
- Lindström, M. in *The Ordovician System* (ed. Bassett, M. G.) 501–522 (University of Wales Press, Cardiff, 1976).
- Scotese, C. R. *et al. J. Geol.* **87**, 217–277 (1979).
- Cooper, G. A. & Arellano, A. R. V. *Smithsonian Misc. Coll.* **119**, 1–183 (1952).
- Longoria, J. F. & Gonzales, M. A. *Bol. Dept. Geol. Univ. Sonora* **2**, 106–149 (1979).
- Eells, J. L. thesis, San Diego State Univ. (1972).
- King, R. E. *Bull. geol. Soc. Am.* **50**, 1625–1727 (1939).
- Noll, J. H. *Geol. Soc. Am. Abstr. Prog.* **13**, 99 (1981).
- Tyler, D. L. thesis, Rice Univ. (1975).
- Cameron, C. S. *Geol. Soc. Am. Abstr. Prog.* **12**, 100 (1980).
- Anderson, T. H. & Silver, L. T. *Geol. Soc. Am. Abstr. Prog.* **13**, 47 (1981).
- Saleeby, J. in *The Geotectonic Development of California* (ed. Ernst, W. G.) 132–181 (Prentice Hall, New Jersey, 1981).
- Gastil, R. G. *et al. Bull. Am. Ass. petrol. Geol.* **57**, 746–747 (1973).
- Gastil, R. G. *et al. in The Geotectonic Development of California* (ed. Ernst, W. G.) 284–305 (Prentice Hall, New Jersey, 1981).
- Beck, M. E. Jr & Plumley, P. W. *Bull. geol. Soc. Am.* **90**, 792–794 (1979).
- Ersine, B. G. & Marshall, C. M. A. *EOS* **61**, 948 (1980).

Musth in the African elephant, *Loxodonta africana*

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The phenomenon of musth in male Asian elephants, *Elephas maximus*, has long been recognized¹. Musth, which has been likened to rutting behaviour in ungulates², refers to a set of physical and behavioural characteristics displayed periodically by adult male elephants. The most obvious manifestations are a sharp rise in aggressive behaviour, copious secretions from and enlargement of the temporal glands, and the continuous discharge of urine³. It has been speculated that a similar phenomenon occurs in males of the African genus, *Loxodonta africana*, but most workers have concluded that it does not exist^{4–7}. Here we show that musth does occur in the African elephant and that its manifestations are similar to those in the Asian elephant.

Musth, well documented in the domestic and wild Asian elephant^{2,3,8,9}, is confined to post-pubertal males, is accompanied by high testosterone levels⁸, and usually lasts for 2–3 months, with a range of several weeks to 9 months³. Individual males tend to come into musth annually or biannually, but unlike the seasonal rutting of most ungulates, musth periods are unsynchronized and occur throughout the year, with usually only one male in musth at a time⁹. In wild populations males in musth show a positive association with female herds⁹.

The most important indicator of musth in the Asian elephant is the onset of secretion from the temporal glands, which, with rare exceptions, occurs only in males in this condition^{2,3}. In the African genus, however, temporal gland secretions of short duration often occur in both immature and mature elephants of both sexes. Previous studies have shown that there is no relationship between such secretions and sexual activity^{4,6}. It is perhaps for this reason that many workers have concluded that musth does not occur in the African elephant.

The discussion of musth has been further confused by the incorrect use of the term, which in Africa is often applied to temporal gland secretions alone, rather than to the behavioural and physiological syndrome originally intended in Asia. Thus males, females and juveniles are often referred to as 'having musth' when they are observed with temporal gland secretions. To avoid such confusion we use the term 'musth' in its original sense and the term 'temporin' as used by Sikes⁷ to refer to secretions from the temporal glands.

We have made continuous observations on male African elephants since September 1975, as part of a long-term study of the elephant population in Amboseli National Park, Kenya. The Park covers an area of 390 km² and consists of semi-arid wooded, bushed and open grassland interspersed with a series of permanent swamps. The surrounding area, making up an ecosystem of ~3,500 km², is semi-arid savannah in which water availability is highly seasonal¹⁰. This ecosystem is inhabited by a free-ranging population of elephants numbering 580 individuals, of which ~155 are adult males. All adults of both sexes and most juveniles are known individually.

During the 5 years of data collection, we observed a pattern of characteristics displayed by the older males (those estimated as being >30 years) in the population. We first noticed individuals with continuously dripping urine, which was accompanied by a strong odour and greenish coloration to the proximal part of the penis and the distal part of the sheath. We referred to this as the 'green penis syndrome' or 'GP'. GP in bulls was recorded whenever it was observed together with other accompanying characteristics, which included a pronounced enlargement of and continuous and copious secretions from the temporal

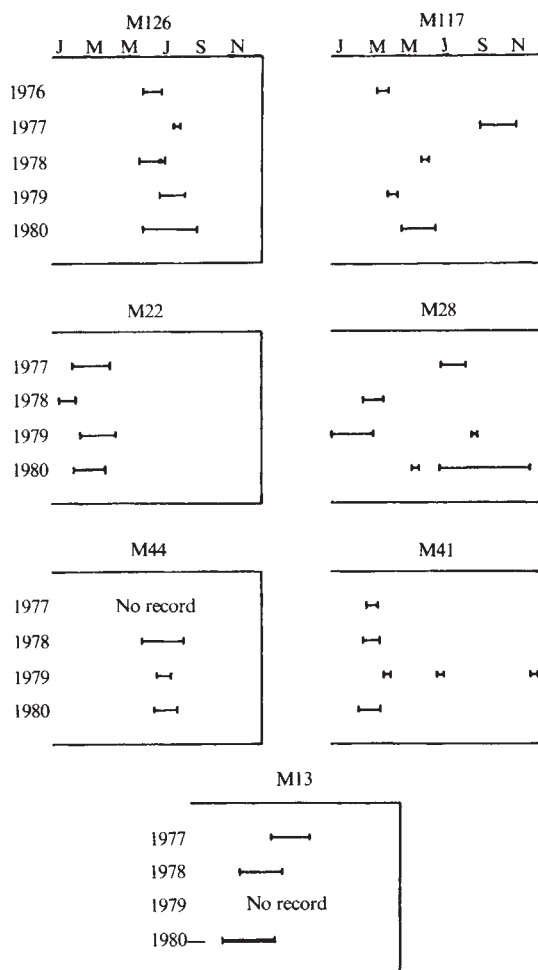


Fig. 1 The minimum duration and degree of periodicity of musth in seven males for the years 1976–1980. The vertical lines indicate the first and last days that the bull was observed to be in musth.

glands, an increase in aggression, and a positive association with female herds. The occurrence of these characteristics, their frequency, duration and association are examined here for seven large bulls in the population for which we have at least 3 years of data.

The coincidence of copious temporin and GP was pronounced in the seven bulls ($\chi^2 = 84.58$, d.f. = 1, $P < 0.001$). There was also a positive correlation between the occurrence of the secretion of both temporin and urine (GP) and the amount of aggressive behaviour shown by these bulls. For each of the seven bulls, we examined the rate of aggressive interactions during secreting and non-secreting periods and found a significant increase in aggression among all bulls who were secreting (Wilcoxon matched-pairs test: $T = 0$, $n = 7$, $P < 0.01$). In addition there was a positive correlation between GP and association with female herds. When the bulls were seen with females, GP was observed in 93% of the sightings ($n = 123$), whereas when they were in groups of bulls alone, GP was observed in only 11% of the sightings ($n = 83$). GP is thus strongly correlated with the association of males with females ($\chi^2 = 141.29$, d.f. = 2, $P < 0.001$).

An examination of the records of frequency and duration of the described characteristics in the seven bulls reveals an individual periodicity which is variable (Fig. 1). Five of the bulls exhibited the characteristics once each year, one twice (M28), and another three times (M41). For some males (M126, M22, M44 and M13), the onset of the phenomenon is fairly consistent from one year to the next, whereas for others (M117, M28 and M41) there is no clear pattern. For all bulls observed exhibiting the characteristics at some point in the study ($n = 18$), the range of duration was from 1 to 103 days.

The similarity between the physical and behavioural characteristics shown periodically by African male elephants and those described as musth for Asian males leads us to conclude that musth does occur in the African genus. Several factors have probably contributed to the fact that musth has been overlooked in the African elephant. In wild populations the presence of temporin in both sexes confused the issue and without long-term records on individually known males musth was not detected. Among captive African males, there are few individuals > 30 years old, because large adults are difficult to handle, thus most would not be old enough to come into musth.

Recent evidence from our study reveals that bulls in musth become sexually more active and achieve a higher dominance rank than at other times, and that musth males are significantly more often in consort relations with oestrous females than are non-musth males. Thus musth may play a part in an individual's reproductive success. Further investigations by J.H.P. are examining the behavioural and ecological factors that determine the age and rank at which males initially come into musth, the timing and duration of musth periods, and how these considerations relate to reproductive success.

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1. Sanderson, G. P. *Thirteen Years Among the Wild Beasts of India* (W. H. Allan, London, 1882).
2. Eisenberg, J. F., McKay, G. M. & Jainudeen, M. R. *Behaviour* **38**, 193–225 (1971).
3. Jainudeen, M. R., McKay, G. M. & Eisenberg, J. F. *Mammalia* **36**, 247–261 (1972).
4. Hanks, J. J. S. *Afr. Wildl. Mgmt. Ass.* **3**, 31–39 (1973).
5. Buss, I. O., Rasmussen, L. E. & Smuts, G. L. *Mammalia* **40**, 437–451 (1976).
6. Short, R. V., Mann, T. & Hay, M. F. *J. Reprod. Fert.* **13**, 517–536 (1967).
7. Sikes, S. K. *The Natural History of the African Elephant* (Weidenfeld and Nicolson, London, 1971).
8. Jainudeen, M. R., Katongole, C. B. & Short, R. V. *J. Reprod. Fert.* **29**, 99–103 (1972).
9. Kurt, F. *IUCN N.S. Publ.* **24**(2), 618–634 (1974).
10. Western, D. E. *Afr. Wildl. J.* **13**, 265–286 (1975).

Formation of ACh receptor clusters induced by positively charged latex beads

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An early event in the formation of neuromuscular junctions in tissue cultures of neurones and muscle cells is an accumulation of acetylcholine receptors (AChRs) in the postsynaptic membrane^{1–5}. As well as direct neuronal contact, AChR cluster formation can also be induced by certain soluble factors extracted or released from neurones^{6–8}, by isolated basal lamina material⁹ or by a piece of degenerating nerve¹⁰. These experiments suggest that certain 'trophic factors' released by the neurones or the neuronal cell surface itself may cause the receptor clustering. On the other hand, stable AChR clusters are also present in pure muscle cultures without neuronal influence^{1,10–13}, which indicates that an interaction between the muscle and an exogenous cue existing on the substrate or in the medium may be sufficient to trigger the mechanism for the clustering of AChRs in the muscle. Here we report the effect of charged latex beads on the formation of AChR clusters in cultured muscle cells. Our results clearly indicate that AChR clusters form specifically at the contacts with polylysine-coated beads. Furthermore, these beads suppressed clusters that formed in the non-contact area before the introduction of the beads.

Table 1 Association of AChR clusters with beads

Experiment	Days of bead-muscle co-culture	Beads	AChR clusters (%)*				No. of clusters scored	No. of cells scored
			Top/edge		Bottom			
			+	-	+	-		
A	2	Polylysine	96	3	0	1	283	22
		Uncoated	12	64	0	24	175	22
		Polycarboxylate	6	73	0	21	188	22
B	1	Polylysine	87	11	0	2	188	20
		Uncoated	8	66	0	26	135	14
B	3	Polylysine	91	9	0	0	286	20
		Uncoated	9	73	0	18	225	20
B	6	Polylysine	94	4	2	0	176	21
		Uncoated	4	60	0	36	109	13

Experiments A and B were from two separate batches of muscle cultures. To minimize any influence on the formation of AChR clusters by an interaction between neighbouring muscle cells or by factor(s) released by the cells, we plated the cells at extremely low density: 20–30 cells per 18×18 mm cover-glass on average. Usually all clusters located on each cell, including its top, edge and bottom, and all cells in each culture were scored. Polylysine beads were prepared from uncoated polystyrene latex beads. Polycarboxylate beads were obtained directly from Polysciences (surface charge, 0.46 mequiv. COO⁻ per g polymer).

* AChR clusters located on the top or the edge of the cell, or on the bottom of the cell as judged by the focusing level of the microscope. Each cluster, after fluorescence observation, was examined with phase-contrast optics to assess its association with beads by simply turning on the transmitted light illuminator without moving the specimen. +, Cluster associated with beads; -, cluster not associated with beads.

Muscle cells isolated from *Xenopus laevis* embryos were cultured on coverslips in Steinberg's solution: 60 mM NaCl, 0.7 mM KCl, 0.4 mM Ca(NO₃)₂, 0.8 mM MgSO₄, 10 mM HEPES, pH 7.4, supplemented with 10% L-15 (Leibovitz) medium and 1% fetal bovine serum^{1,14}. Positively charged beads were made by incubating 1- μ m polystyrene latex beads with 1 mg ml⁻¹ poly-L-lysine (molecular weight (MW) 1,000–4,000; Sigma), dissolved either in distilled water or in phosphate-buffered saline (PBS), overnight at 4°C, then washing thoroughly with distilled water or PBS. Uncoated beads were used as control. Negatively charged 0.9- μ m polycarboxylated beads (Polysciences) were also used. Beads of each type were suspended in culture medium and applied to separate muscle cultures. After incubation for 10–30 min, excess beads were washed off and the cultures transferred to fresh medium. All three kinds of beads brought into contact with the cells adhered strongly to them, even when subjected to repeated washing, as shown in Fig. 1. At the stage required, the cultures were labelled with tetramethyl rhodamine-conjugated α -bungarotoxin (R-BTX), which binds specifically to AChRs^{1,15,16}. After washing and fixing with 95% ethanol at -20°C, the culture was mounted on a slide with a mixture of polyvinyl alcohol and glycerol and examined using a Leitz Orthoplan microscope equipped with an epifluorescence illuminator. The location of fluorescent R-BTX-bound AChR clusters was correlated with the phase-contrast image of the cell and the beads by switching between epifluorescence and phase-contrast optics. Fluorescence of AChR clusters was completely suppressed when the cultures were first incubated with native α -BTX then with R-BTX.

In 1-week control cultures (without beads), an average of eight AChR clusters (0.5 μ m to >10 μ m) per cell were observed. Most of these (60–80%) were observed on the top or along the edge of the cell but 20–40% were also observed on the bottom of the cell. A class of large clusters similar to the one shown in Fig. 2a was observed in ~50% of the cells. These large clusters consisted of many smaller AChR aggregates and were found predominantly on the bottom of the cell, in contact with the substrate. In cultures treated for 1–2 days with uncoated or polycarboxylated beads, the distribution of AChR clusters was similar to that of the control. There was no significant association between the beads and the AChR clusters (Fig. 2a–d, Table 1A). About 45% of these cultured cells had large bottom-located clusters (see Fig. 2a). However, in cultures treated with polylysine-coated beads for 1–2 days, almost all AChR clusters observed by R-BTX fluorescence were associated with single beads or small aggregates of beads which seemed to be in contact with the muscle cell. The results of one set of experiments are given in Table 1A and representative examples shown in Fig. 2e–h. It is obvious from Table 1A and B that almost all AChR

clusters in polylysine bead-treated cultures are located on the top or the edge of the cell where the beads come into contact with the cell. The absence of AChR clusters on the bottom of the cell corresponds well with the absence of beads in that area, from which they are excluded by the narrow gap between the cell and the substrate. This is in contrast to the control cultures and those treated with uncoated or polycarboxylated beads which usually show a substantial number of bottom clusters, particularly the large type illustrated in Fig. 2a. This indicates that the polylysine-coated beads not only induce the formation of new AChR clusters at bead-muscle contacts but also suppress the pre-existing clusters in non-contact areas.

In these experiments we used a dilute bead concentration to reduce the possibility of random association of beads with existing receptor clusters. As the size and shape of the muscle cells are heterogeneous, the number of bead-muscle contacts per cell also shows a wide variation. However, from Fig. 3 it is clear that the number of bead-muscle contacts is positively correlated with the number of associated AChR clusters, which further supports the notion that the beads induce formation of new clusters. There were ~30% apparent bead-muscle contacts not associated with receptor clusters. Clusters formed around single beads were discrete and closely apposed to the beads (Fig. 2e–h) whereas those formed under an aggregate of beads were usually composed of several discrete sub-clusters (Figs 2g–h and 4a–b).

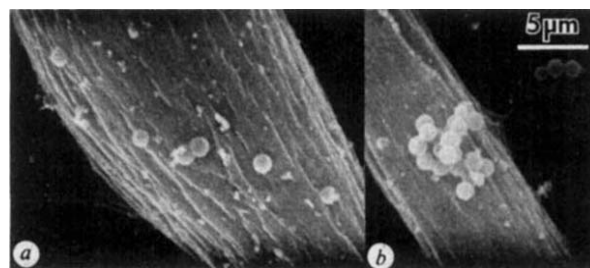
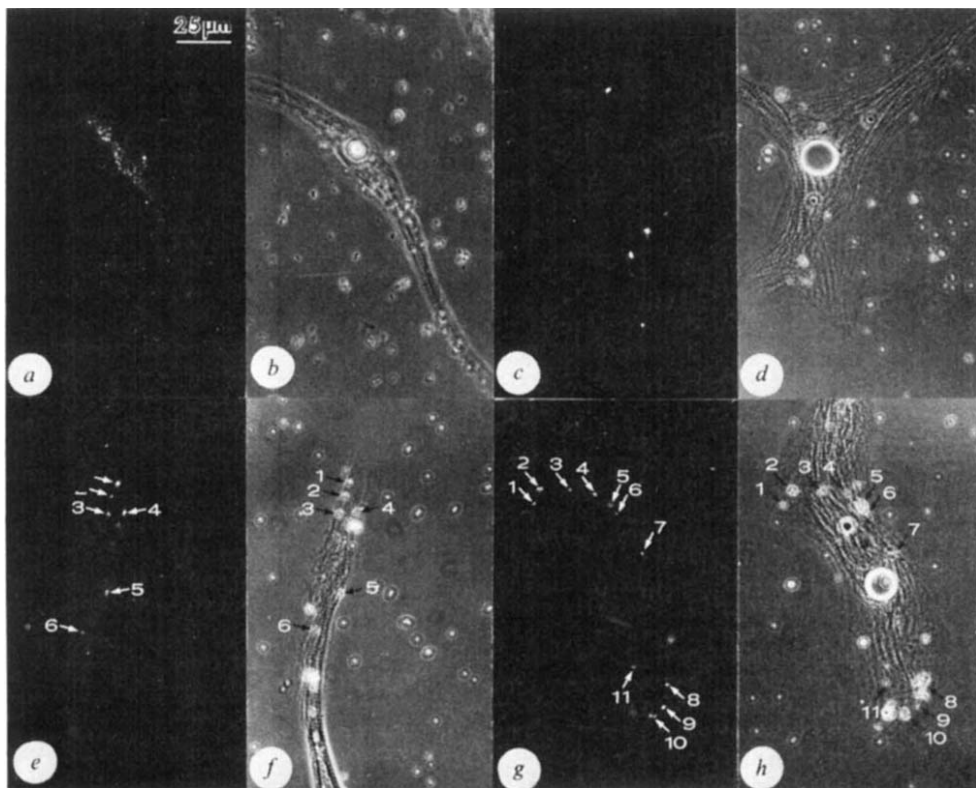


Fig. 1 Scanning electron micrograph of the association of latex beads with muscle cells. Poly-L-lysine-coated beads were used in this experiment. Two days after the bead-muscle co-culture, the cells were fixed using a mixture of 3% glutaraldehyde and 4% tannic acid, followed by post-fixation with 1% OsO₄. The preparation was then dehydrated in a graduated ethanol series, critical-point dried through CO₂, coated with palladium-platinum on a sputter coater and viewed with a JEOL 35C scanning electron microscope at an accelerating voltage of 25 kV. The attachment of single beads (a) or small aggregates of beads (b) to muscle cells can be clearly seen. This bead-muscle association is obviously strong enough to withstand the various medium changes during specimen preparation.

Fig. 2 Relationship between the AChR clusters and the bead-muscle contacts. The cells were treated with latex beads for various time periods and the location of AChR clusters assayed by labelling with R-BTX and fluorescence microscopy after fixation with cold 95% ethanol. A Leitz Orthoplan microscope equipped with a $\times 40$ phase-contrast objective (n.a. 1.3) and an epifluorescence illuminator with filter cube N2.1 was used. *a, c, e* and *g* are fluorescence micrographs; *b, d, f* and *h* the corresponding phase-contrast images. *a, b*, Cells were treated with polycarboxylated beads for 2 days. In *a*, the focus was on the bottom of the cell in contact with the substrate. In this area, the beads are excluded. A large AChR cluster, composed of many smaller subunits, is seen. This type of cluster was observed in about 50% of the cultures treated with polycarboxylated beads or uncoated beads as well as in control cultures without beads; *b* focuses on the centre of the cell. *c, d*, Cells were treated with uncoated beads for 2 days; *c* focuses on the AChR clusters on the top of the cell where the beads are also located (*d*). It is clear that the AChR clusters do not bear any particular relationship to the beads. *e-h*, Cells were treated with poly-L-lysine-coated beads for 1 day (*e, f*) and 2 days (*g, h*). In *e*, most of the AChR clusters located on the top and the edge of the cell are found precisely at the bead-muscle contacts. The numbers denote the correspondence in location between the fluorescence (*e*) and phase-contrast (*f*) images obtained using identical focus. Beads which fall on the glass substrate are not associated with fluorescence. In *g* and *h*, a larger number of bead-muscle contacts is also associated with a larger number of bead-associated AChR clusters. Both single beads and small aggregates of beads (nos 5, 8-10) can cause AChR cluster formation.



Clusters formed shortly after the beads came into contact with the muscle cell; even after 6 h of bead-muscle co-culture, we detected 65% of the clusters already associated with beads. Table 1B summarizes the results of a time study using polylysine-coated and uncoated beads. It shows that almost a full effect of the polylysine-coated beads was evident after only 1 day of bead-muscle co-culture. The receptor clusters formed at

the contacts seemed to be stable for at least the 6 days of this study. No R-BTX fluorescence was ever detected when beads came into contact with the culture substrate (Fig. 2*e-h*) or non-muscle cells (Fig. 4*c-d*).

These results indicate that the effect of polylysine-coated beads is very similar to that of nerve innervation. During the formation of a neuromuscular synapse *in vitro*, the nerve induces AChR cluster formation at the innervated site and suppresses pre-existing clusters in non-innervated areas². Our data indicate that a localized interaction between the muscle surface and polylysine-coated beads can trigger the mechanism for the formation of AChR clusters. This process may be mediated by the positive charge on the surface of the beads, a chemical interaction between the polylysine molecules and the cell surface, or even certain factor(s) from the culture medium adsorbed onto the positively charged beads. Further experiments are being done to investigate these possibilities.

We have repeated the above experiments using larger (6 μ m) polylysine-coated beads, which make larger contacts with the muscle cells and the resultant AChR clusters are also larger, but each cluster is still located within the confines of the contact area. This further indicates that a local cell-bead interaction triggers the clustering of AChRs. Although latex beads are known to be phagocytosed by certain cultured cells, for example, macrophages¹⁷, recent electron microscopic (EM) studies (H.B.P., P.-C.C. and P.W.L., in preparation) give no evidence of this in cultured muscle cells. Also, in our scanning electron micrographs (Fig. 1), beads were always seen on the cell surface and no intermediate stages of phagocytosis were observed. We have found that the bead-induced AChR clusters are accompanied by membrane-associated cytoplasmic densities in thin sections and clusters of large intramembranous particles in freeze-fracture replicas, which are also characteristic of spontaneously formed AChR clusters³. Closely related to our

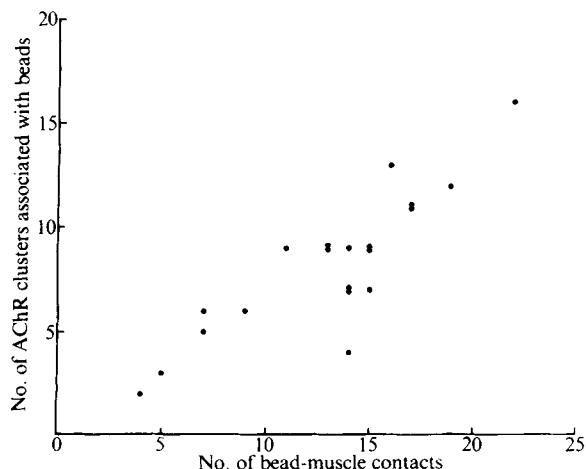


Fig. 3 Correlation between the number of AChR clusters associated with polylysine-coated beads and the number of bead-muscle contacts on individual cells. Each of the 20 cells scored is represented by a black circle. The number of bead-associated AChR clusters increases approximately linearly with the number of bead-muscle contacts.

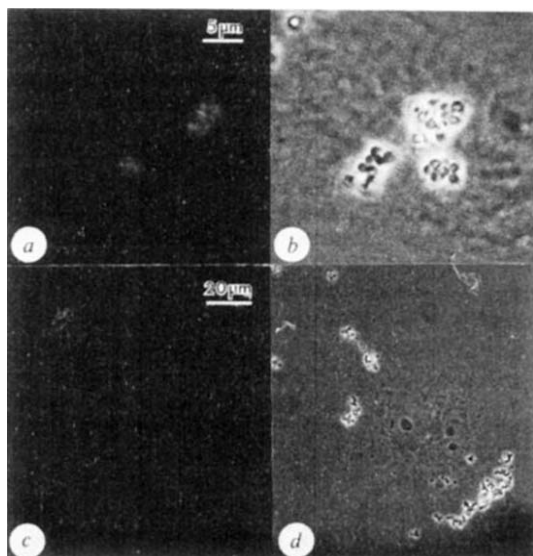


Fig. 4 *a, b*, AChR clusters formed at the bead-muscle contacts shown at a higher magnification than in Fig. 2. The small subunits in a cluster associated with multiple beads are obvious. *c, d*, The contact area between the bead and the non-muscle cell is not associated with any R-BTX fluorescence. *a, c*, R-BTX fluorescent images; *b, d*, phase-contrast images. These two cells were from the same culture and thus received the same treatment.

observation is the finding¹⁸ that neurites in cell cultures of rat cerebellum can form apparent presynaptic elements, including dense material and accumulation of synaptic vesicles, at their contacts with positively charged Sepharose beads. Thus a surface interaction between the neurite and the target cell may trigger the formation and registration of both the pre- and postsynaptic specialization during the initial stages of synaptogenesis.

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- Anderson, M. J., Cohen, M. W. & Zorychta, E. *J. Physiol., Lond.* **268**, 731-756 (1977).
- Frank, E. & Fischbach, G. D. *J. Cell Biol.* **83**, 143-158 (1979).
- Peng, H. B., Nakajima, Y. & Bridgman, P. C. *Brain Res.* **196**, 11-31 (1980).
- Cohen, M. W. & Weldon, P. R. *J. Cell Biol.* **86**, 388-401 (1980).
- Kidokoro, Y., Anderson, M. J. & Gruener, R. *Dev. Biol.* **78**, 464-483 (1980).
- Podleski, T. R. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 2035-2039 (1978).
- Christian, C. N. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4011-4015 (1978).
- Jessell, T. M., Siegel, R. E. & Fischbach, G. D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5397-5401 (1979).
- Rubin, L. L., Gordon, A. S. & McMahan, U. J. *Soc. Neurosci. Abstr.* **6**, 330 (1980).
- Jones, R. & Vrbova, G. *J. Physiol., Lond.* **236**, 517-538 (1974).
- Sytkowski, A. J., Vogel, Z. & Nirenberg, M. W. *Proc. natn. Acad. Sci. U.S.A.* **70**, 270-274 (1973).
- Fischbach, G. D. & Cohen, S. A. *Dev. Biol.* **31**, 147-162 (1973).
- Bekoff, A. & Betz, W. J. *Science* **193**, 915-917 (1976).
- Peng, H. B. & Nakajima, Y. *Proc. natn. Acad. Sci. U.S.A.* **75**, 500-504 (1978).
- Lee, C. Y. A. *Rev. Pharmac.* **12**, 265-286 (1972).
- Ravdin, P. & Axelrod, D. *Analyt. Biochem.* **80**, 585-592 (1977).
- Walter, R. J., Berlin, R. D., Pfeiffer, J. R. & Oliver, J. M. *J. Cell Biol.* **86**, 199-211 (1980).
- Burry, R. W. *Brain Res.* **184**, 85-98 (1980).

Creation of direction selectivity in adult strobe-reared cats

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Animals raised in a stroboscopically illuminated environment have deficits in several visual functions, including visuo-motor integration¹, discrimination learning² and spatial contrast sensitivity³. Moreover, recordings from the visual pathways of strobe-reared animals show severe functional abnormalities, including greatly reduced selectivity for orientation and for directional motion in neurones of the visual cortex and superior colliculus⁴⁻⁹. Subsequent normal visual experience improves cortical orientation selectivity, but does not alter the neural deficit in direction selectivity^{6,7}. As the motion-analysing capacities of strobe-reared animals have not been studied, we examined the ability of strobe-reared cats to discriminate stationary from moving patterns. We report here that the cats detected motion in the direction for which they had originally been trained much better than motion in other directions. In recordings from striate cortex in these animals, orientation and direction-selective neurones were encountered with a frequency much higher than that seen in strobe-reared cats not trained in motion discrimination, and comparable with that in normal cats. Moreover, the distribution of the preferred directions of these neurones was sharply biased towards the direction first seen in training. We conclude that there exists an extended period of cortical plasticity in strobe-reared animals, which, in contrast to that previously reported⁶, includes plasticity of direction selectivity.

We carried out behavioural tests on five cats; two were reared from birth to the age of at least 14 months in a room illuminated for 12 h each day with a stroboscopic flash of 3 μ s duration at a rate of 0.67 Hz, and two were raised normally. One was raised in an environment intermittently illuminated with a flash of light 750 ms in duration at 0.67 Hz; the luminance of this flash was adjusted so that normal adult cats could resolve fine detail in this illumination as well as they could in the stroboscopic illumination. Behavioural testing began no less than 4 months after the animals were removed from their rearing environment.

The cats were trained to discriminate stationary from moving random-dot patterns using a forced-choice procedure based on that developed by Berkley¹⁰; our modification of this method has been described in detail elsewhere¹¹. The animals were trained to indicate which stimulus moved by pressing with their noses on one of two transparent panels through which they viewed the stimuli. Each stimulus consisted of a sheet of 400 bright dots, each 0.5° in diameter, on a dark background; the sheet subtended 22° at a viewing distance of 30 cm. Most of the energy in the pattern was concentrated at spatial frequencies below 1c/deg, which are visible to strobe-reared cats¹¹.

During the initial training, the stimulus always moved to the right at 44° s⁻¹; after 12-30 days, with 200 trials per day, all cats satisfactorily discriminated between this stimulus and the stationary one. Strobe-reared cats were no slower in acquiring this discrimination than control cats. We next measured the lowest detectable speed using the method of constant stimuli. In each session five speeds were chosen to bracket threshold, and were presented randomly in blocks of five trials. Threshold was taken as the point at which resulting psychometric functions produced 75% correct performance.

The motion thresholds of the control cats improved over several months of testing from an initial value between 8 and 11° s⁻¹ to an asymptotic value between 0.9 and 1.5° s⁻¹. The initial thresholds of the strobe-reared cats were high in comparison (between 17 and 25° s⁻¹); after prolonged testing (~8 months during which the animals received ~18 h of exposure to moving dots) the thresholds stabilized near 3° s⁻¹, 2-3 times higher than those of control cats.

In all tests described above, rightward motion was used. We next examined the transfer of this training to other directions.

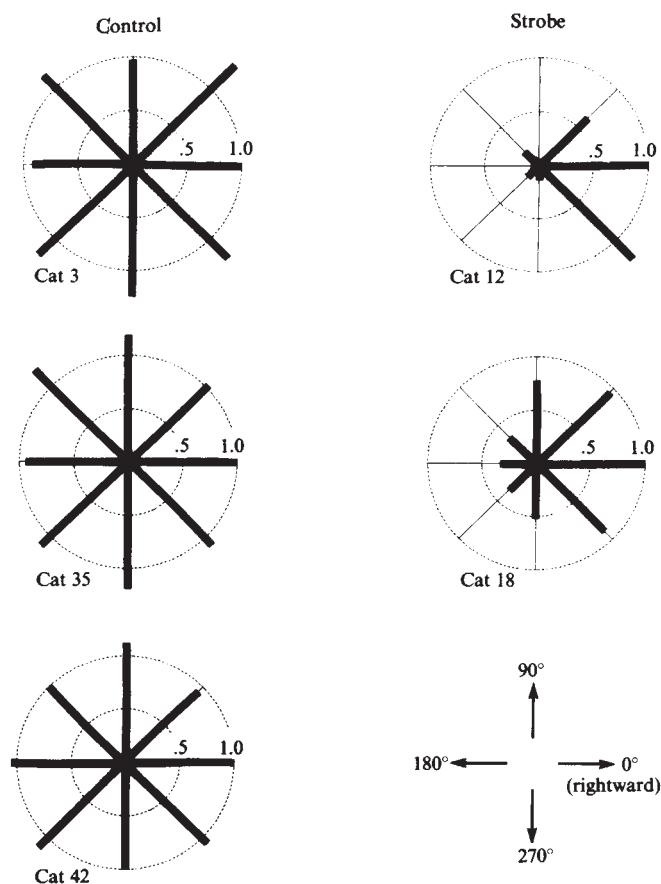


Fig. 1 Polar diagrams showing the speed sensitivity of control and strobe-reared cats to motion in eight directions. Each bar plots the inverse of the speed required for 75% correct performance on a two-choice discrimination between a stationary and a moving random-dot field. The bars in each case are normalized so that sensitivity to rightward motion is given a value of 1. Data are shown for two normal cats (3 and 35), one cat raised in an intermittently illuminated environment (42, see text) and two cats raised in a stroboscopically illuminated environment (12 and 18). Their actual thresholds for rightward motion were 0.9, 1.2, 2.0, 2.0 and 4.4° s⁻¹, respectively. A repeated measures analysis of variance showed that none of the control animals was significantly better at motion discrimination in any particular direction ($P > 0.05$). However, both strobe cats were significantly more sensitive to motion within 45° of rightward than they were to other directions ($P < 0.05$).

On each day, the threshold for one direction was tested. Over several days, an irregular sequence of eight directions, evenly spaced around 360°, was presented. Figure 1 shows the sensitivity of each of the five cats to motion as a function of the direction tested. In each polar diagram, the length of bar represents the inverse of the speed threshold for a particular direction of motion; the values are normalized with respect to the cat's sensitivity to rightward motion, which is given a value of 1. The control cats had a similar sensitivity in all test directions, whereas the strobe-reared animals were much more sensitive to rightward motion than they were to motion in directions more than 45° from this, the initial direction of training. The high thresholds for directions for which no training had been given remained stable over 7 months of further training, in which stimuli moved upwards, downwards and to the left, and did not improve as the initial high thresholds for rightward motion had done earlier. Note also that this deficit did not represent a simple failure to generalize from one direction to another, as the strobe-reared cats were capable of discriminating motion in all directions, yielding stable psychometric functions of normal slope; it was simply that when tested with rightward motion, they required only low stimulus speeds compared with those required for detection of motion in other directions.

The peculiar sensitivity of these strobe-reared cats to rightward motion led us to examine the orientation and direction selectivity of striate cortical units, using methods described elsewhere¹². Control data were obtained from six other adult strobe-reared cats that had received comparable periods of normal visual experience but had not been trained to discriminate motion, and from seven normally reared adult cats. Animals were prepared for electrophysiology using barbiturate anaesthesia (Pentothal); they were then paralysed with Flaxedil and artificially ventilated with 80% N₂O in O₂ and CO₂. Their corneas were covered with contact lenses containing 4-mm artificial pupils, and supplementary lenses were used to focus the eyes on a screen 72 cm distant. Single cortical units were isolated using tungsten microelectrodes, and their activity amplified and displayed. We studied the properties of 88 and 87 units in the two strobe-reared animals, in each case drawing our samples equally from long medially directed penetrations in the two hemispheres. For comparison, we made recordings of 183 units from normal cats. All units were histologically verified to lie in area 17.

Receptive fields were mapped on a tangent screen using bars, edges and spots of light, and classified according to the scheme of Hubel and Wiesel¹³ as modified by Blakemore and Van Sluyters¹⁴. No quantitative response measures were used but we paid careful attention to the orientation and direction selectivity of each neurone. Cells were classified as orientation sensitive (OS), orientation biased (OB), or not oriented (NO) using criteria described elsewhere¹⁴. Cells were direction selective (DS) if they responded markedly better to one of the two directions of motion of an optimally oriented stimulus, direction biased (DB) if they responded discriminably better to one direction than the other, and not directional (ND) if there was no difference in the responses to the two directions.

The data from the two cats trained to discriminate motion were generally similar to those from normal cats, and differed markedly from those of other strobe-reared animals studied by us and others^{4-7,15}. Table 1 lists the proportions of OS and OB neurones in the three groups of cats, and the proportions of those in each of the three classes of direction sensitivity. While strobe-reared cats given a period of normal visual experience showed abnormally low proportions of orientation- and direction-sensitive neurones (62% and 35% respectively), both motion-trained animals had roughly normal proportions of selective cells: in our sample, 93% of neurones were classified as OS or OB; of these 65% were either DS or DB. By comparison, 99% of neurones in normal cats were orientation sensitive, and 74% of these were DS or DB.

While cells preferring all orientations were direction selective in the motion-trained cats, there was a marked bias in the

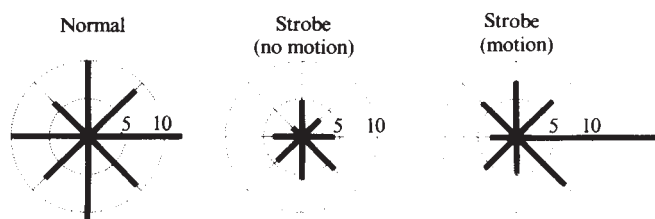


Fig. 2 Polar histograms showing the distributions of direction preference for cortical neurones showing direction sensitivity in three groups of cats: normal; strobe-reared but not motion-trained; and strobe-reared and trained in motion discrimination (cats 12 and 18 from Fig. 1). The histograms show the percentage of the total population of cortical neurones in each group that preferred directions in the ranges indicated. The results are based on totals of 192 cells from seven normal cats, 183 from six strobe-reared cats not trained in motion discrimination, and 175 cells from two strobe-reared cats trained in motion discrimination using rightward motion. The distributions of direction preference were similar in the two motion-trained cats: 21% of all cells in cat 18 and 17.5% in cat 12 preferred rightward motion, whereas only 5% of cells in cat 18 and 2% in cat 12 responded preferentially to leftward motion.

Table 1 Proportion of orientation- and direction-sensitive neurones

Experimental group	Cells	All cells					OS and OB cells				
		%OS	%OB	%DS	%DB	%ND	%OS	%OB	%DS	%DB	%ND
Normal cats	192	90	9	42	32	26					
Strobe cats (not motion-trained)	183	40	22	12	23	65					
Strobe cats (motion-trained)	175	91	2	32	33	35					

distribution of direction selectivity. This is shown in Fig. 2, which gives the direction preferences of cortical units from the three groups of cats. In the two motion-trained cats, 59 units had preferred orientations within 22.5° of vertical: of these 40 (68%) showed a direction preference, with 34 of the 40 (85%) preferring movement to the right and only six (15%) preferring movement to the left. In these cats, 89 cells were both direction selective and preferred orientations within 67.5° of vertical; 62 of these (70%) preferred the direction having a rightward component; only 27 (30%) preferred motion with a leftward component. By comparison, among the 70 direction-sensitive cells preferring orientations within 67.5° of horizontal, 36 (51%) preferred motion with an upward component and 34 (49%) preferred motion with a downward component. Neither the direction-sensitive neurones from normal cats nor the few such neurones from strobe-reared cats not motion-trained showed any significant anisotropy in the distributions of their preferred directions.

Thus in two adult strobe-reared cats trained extensively to discriminate motion, we found both recovery of motion detection performance and recovery of cortical direction selectivity. This confirms previous suggestions^{6,16,17} that in strobe- and dark-reared cats it is possible to demonstrate a period of cortical plasticity that extends beyond the traditional 'sensitive period' for cortical development. Our results are unusual in that they suggest that this extended plasticity, which does not normally include direction selectivity, may do so if animals are preferentially exposed to moving stimuli.

Also interesting is our finding of a bias towards the initially trained direction in both the psychophysical motion sensitivity and the directional preferences of cortical neurones in these cats. Cortical direction selectivity can be biased in young kittens exposed to a restricted range of directions of motion¹⁸⁻²⁰, but these changes have only been shown to occur during a restricted period in early life that ends well before the period of sensitivity to the effects of monocular occlusion^{21,22}. Our data show that a qualitatively and quantitatively similar effect may be seen in adult strobe-reared cats exposed to a motion-biased environment. The magnitude of the effects is similar to those reported elsewhere for young animals, which is surprising in view of the fact that our animals received 4 months of normal vision before training and received 12 h per day of concurrent unbiased visual stimulation. In our experiments the cats were required to attend to the moving stimuli to obtain behavioural reward, which may have rendered those stimuli in some way more potent modifiers of cortical function.

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- Hein, A., Gower, B. C. & Diamond, R. M. *J. comp. physiol. Psychol.* **73**, 188-192 (1970).
- Chalupa, L. M. & Rhoades, R. W. *Expl Neurol.* **61**, 442-454 (1978).
- Pasternak, T. & Merigan, W. H. *Nature* **280**, 313-314 (1979).
- Cynader, M., Berman, N. & Hein, A. *Proc. natn. Acad. Sci. U.S.A.* **70**, 1353-1354 (1973).
- Olson, C. & Pettigrew, J. D. *Brain Res.* **70**, 189-204 (1974).
- Cynader, M., Berman, N. & Hein, A. *Expl Brain Res.* **25**, 139-156 (1976).
- Cynader, M. & Chernenko, G. *Science* **193**, 504-505 (1976).

- Orban, G., Kennedy, M., Maes, H. & Amblard, B. *Archs ital. Biol.* **116**, 413-419 (1978).
- Flandrin, J. H., Kennedy, H. & Amblard, B. *Brain Res.* **101**, 576-581 (1976).
- Berkley, M. A. in *Animal Psychophysics* (ed. Stebbins, W. J.) 231-247 (Appleton-Century-Crofts, New York, 1970).
- Pasternak, T. & Merigan, W. H. *J. comp. physiol. Psychol.* **94**, 943-952 (1980).
- Movshon, J. A. *J. Physiol., Lond.* **261**, 125-174 (1976).
- Hubel, D. H. & Wiesel, T. N. *J. Physiol., Lond.* **160**, 106-154 (1962).
- Blakemore, C. & van Sluyters, R. C. *J. Physiol., Lond.* **248**, 663-716 (1975).
- Pasternak, T. & Movshon, J. A. *Invest. ophthalmol. vis. Sci. Suppl.* **225** (1980).
- Cynader, M. & Mitchell, D. E. *J. Neurophysiol.* **43**, 1026-1040 (1980).
- Timney, B., Mitchell, D. E. & Cynader, M. *J. Neurophysiol.* **43**, 1041-1054 (1980).
- Cynader, M., Berman, N. & Hein, A. *Expl Brain Res.* **22**, 267-280 (1975).
- Tretter, F., Cynader, M. & Singer, W. *Brain Res.* **84**, 143-149 (1975).
- Daw, N. W. & Wyatt, H. J. *J. Physiol., Lond.* **257**, 155-170 (1976).
- Berman, N. & Daw, N. W. *J. Physiol., Lond.* **265**, 249-259 (1977).
- Daw, N. W., Berman, N. & Ariel, M. *Science* **199**, 565-567 (1978).

Neuronal precursor cells in the chick neural tube express neurofilament proteins

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Biochemical and immunological differences have been demonstrated in intermediate-sized (or 10-nm) filaments (IFs) from a variety of cell types (for reviews see refs 1-4). Neurofilaments, the IFs of neurones (NIF), are composed of three proteins that differ from those of non-neuronal IFs⁵⁻¹². Yet, in the 2-day-old chick neural tube, virtually all the replicating neuroepithelial cells, the precursor population to spinal cord neurones and glia, contain IF proteins that are immunologically and biochemically identical to the major IF protein present in chick fibroblasts, FIF protein (vimentin¹³, decamin¹⁴); they contain neither NIF proteins nor the IF proteins characteristic of astrocytes (glial fibrillary acidic protein^{15,16}), muscle cells (desmin¹⁷, skeletin¹⁸) and epithelial cells (prekeratin^{19,20}). As the neuronal progeny of these cells mature, they synthesize NIF proteins and cease expression of FIF protein^{10,21}. In this study, we have used antisera against different IF proteins to show that, at a time when neurones are withdrawing from the cell cycle, NIF proteins are present in a small percentage of replicating neuroepithelial cells, probably appearing during the terminal cell cycle of the neuronal precursor; and that for a brief period the postmitotic neuroblast expresses both NIF and FIF proteins.

Our findings are based on immunofluorescent localization of IF proteins using previously characterized antisera against FIF protein^{22,23}, the 180,000-molecular weight (MW)^{10,21} and 70,000-MW²¹ NIF proteins, and IF proteins characteristic of astrocytes²¹ and muscle cells^{23,24}. Cryostat sections were taken primarily from the brachial region of the embryonic chick neural tube, although more rostral regions of the nervous system were also observed. Two stages of embryonic development were examined: Hamburger and Hamilton's²⁵ stage 13 (48-52 h of incubation), before significant neuronal birth, and stage 18 (3 days of incubation), when many neurones are withdrawing from the cell cycle^{26,27}. Both stage 13 and 18 embryos were treated with demecolcine for 4 h before they were killed, as described elsewhere²¹.

Before neuronal birth, the cells of the neural tube form a pseudostratified columnar epithelium—the neuroepithelium. They are a population of replicating cells that contain the precursors to neurones, glial cells and ependymal cells. During mitosis the cells lose their basal attachment to the external limiting membrane, assume a round shape adjacent to the neurocoel and undergo cytokinesis (see ref. 28). Treatment of the embryo with demecolcine arrests mitotic cells in metaphase and causes the IFs to aggregate and form a cable, or ring, around the cell's condensed chromatin. The IF in interphase cells also aggregate into cables in the presence of demecolcine, but these cells do not lose their bipolar shape and the IF cables are predominantly located in the basal processes. In this manner, treatment with demecolcine facilitates detection of small

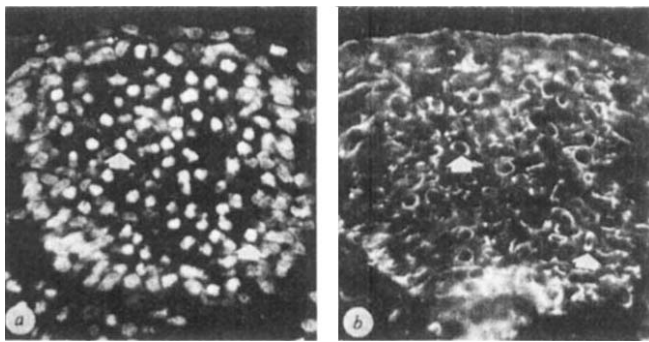


Fig. 1 Two exposures of a single microscopic field in a transverse section through the neural tube of a demecolcine-treated stage 13 embryo after staining with *a*, the fluorescent chromosome stain Bisbenzamid-H (Hoechst, H3325), and *b*, antiserum to FIF protein, localized by indirect immunofluorescence with a rhodamine-conjugated secondary antibody. Tissue preparation and immunofluorescence were performed as previously described²¹. Appropriate microscope filters allow separate visualization of the bisbenzimidazole and rhodamine. The metaphase cells appear as brightly staining areas of condensed chromatin (arrows in *a*). Associated with each of the metaphase cells is a ring labelled with anti-FIF (corresponding arrows in *b*). $\times 140$.

amounts of IF proteins by immunofluorescence, and also allows the unambiguous association of IF aggregates with a specific metaphase cell. As we reported previously²¹, virtually all the metaphase cells in the stage 13 neural tube have FIF cables (Fig. 1), as determined by a fluorescent chromosome stain combined with immunofluorescent labelling using antiserum to FIF protein. None of these cables bind antibodies against the 70,000- or 180,000-NIF proteins, or the IF proteins characteristic of astrocytes or muscle cells.

In the stage 18 neural tube, as in stage 13, almost all ($\sim 98\%$) the metaphase cells contain FIF cables. Unlike stage 13, however, some cells contain cables that bind antibodies to the 70,000- and 180,000-MW NIF proteins. Interphase nuclei positioned in the developing mantle layer have NIF-positive processes, presumably representing axonal formation and NIF synthesis in postmitotic neuroblasts. In addition, we were surprised to find that a small percentage ($\sim 0.75\%$) of the metaphase cells surrounding the neurocoel also have NIF-positive rings (Fig. 2) and must therefore represent cells that have initiated synthesis of NIF before terminal mitosis. Furthermore, outside the neural tube, a population of cells in the vicinity of the otic vesicle is remarkable in that it has a high percentage of metaphase cells with NIF-positive rings (Fig. 3).

Double labelling of sections using antibodies against FIF and NIF shows that metaphase cells with NIF cables also contain FIF, although not always in the same distribution (Fig. 4*a, b*). Similarly, NIF-positive cables associated with interphase nuclei in the ventricular zone also have FIF (Fig. 4*c, d*). While some neuronal processes in the marginal layer of the stage 18 neural tube stain with both anti-NIF and anti-FIF, others bind only anti-NIF. At later stages all neuronal processes have no FIF and contain only NIF²¹.

These findings indicate a definite pattern of IF protein expression during neurogenesis in the spinal cord: (1) replicating neuroepithelial cells contain only FIF protein until the terminal cell cycle; (2) immediately before the final division the neuronal precursor cell begins to synthesize NIF proteins; (3) the post-mitotic neuroblast expresses both NIF and FIF for a short period; and (4) with neuronal maturation, FIF expression ceases and only NIF proteins are detectable^{10,21}.

The absence of NIF at embryonic stages before neuronal birth and the *de novo* appearance of NIF in replicating cells at a stage when neurones are withdrawing from the cell cycle indicates that the neuronal precursor cell initiates NIF synthesis during its terminal cell cycle. In this regard, molecular expression of neuronal differentiation occurs at least by the final S/G2 period of the precursor cell. This is consistent with the appearance of

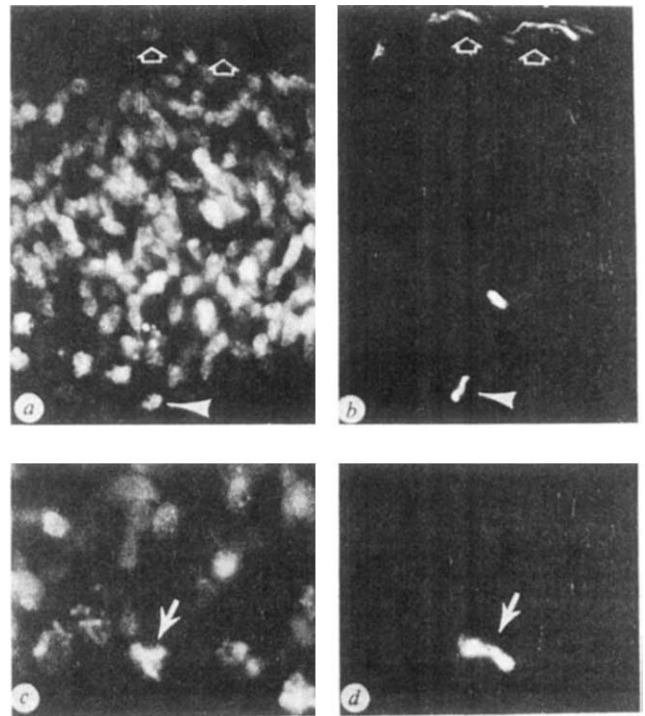


Fig. 2 Each pair of photographs shows one microscopic field of a section from a demecolcine-treated stage 18 neural tube stained with bisbenzimidazole (*a, c*) and antibodies to the 70,000-MW NIF protein (*b, d*). *a, b*, Interphase nuclei in the developing mantle layer (arrows) have associated NIF-containing processes. A metaphase cell (arrowhead) adjacent to the neurocoel has an NIF-positive ring lying perpendicular to the plane of section. NIF proteins are also associated with an interphase cell further from the neurocoel. $\times 280$. *c, d*, Arrow indicates a metaphase cell adjacent to the neurocoel with an associated NIF-positive ring. Note that many metaphase cells do not have NIF-positive rings. $\times 840$.

argentophilic filaments in replicating cells of the embryonic chick retina²⁹. In the central nervous system, in contrast to NIF proteins, other 'markers' of neuronal differentiation appear only in postmitotic neurones. G_{M1} ganglioside³⁰, neurone-specific enolase³¹ and adrenergic characteristics³² are not found in replicating neuronal precursors. However, replicating precursor cells of sympathetic neurones exhibit several characteristics of adrenergic neurones (that is, formaldehyde-induced fluorescence, dense-core vesicles and noradrenaline uptake)^{32,33}, suggesting that neuronal precursors derived from neural crest might express neuronal properties for one or more generations before the terminal cell cycle. In this regard, the high percentage of replicating cells with NIF cables in the vicinity of the otic vesicle may indicate that some neuronal precursor populations

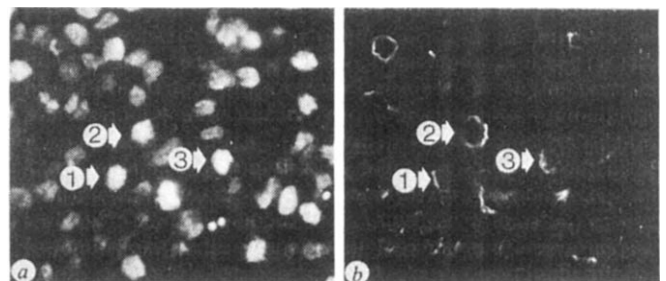


Fig. 3 A transverse section through a region postero-lateral to the otic vesicle from a demecolcine-treated stage 18 embryo stained with (*a*) bisbenzimidazole and (*b*) antibodies to the 70,000-MW NIF protein. A high percentage of metaphase cells in this region have associated NIF-positive rings. The numbered mitotic cells in *a* correspond to the numbered rings in *b*. $\times 380$.

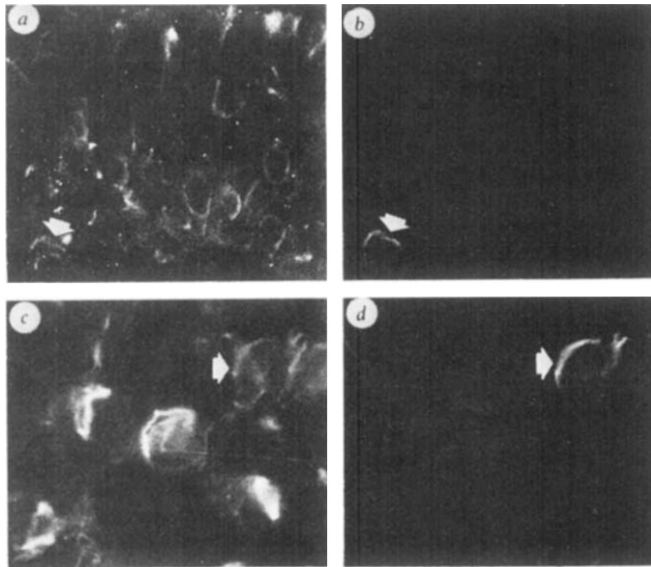


Fig. 4 Photographs of a demecolcine-treated stage 18 neural tube that has been double labelled with antibodies to FIF protein (a, c) and antibodies to either the 180,000-MW NIF protein (b) or the 70,000-MW NIF protein (d). a, b, Although most cells contain FIF, one (arrow) apparently has two cables, one mixed FIF and NIF cable and one pure FIF cable. $\times 560$. c, d, Again, most cells contain FIF, but one cell migrating through the ventricular zone contains both NIF and FIF (arrow). $\times 840$.

synthesize NIF for several generations. Alternatively, these cells may represent a neuronal population synchronously undergoing their terminal mitosis. Although the origin and fate of these cells remains to be determined, it is possible that they are derived from neural crest.

Finally, it is worth stressing that the early appearance of NIF reported here may represent a developmental sequence restricted to only certain types of neurones and may not apply to all neurones of the nervous system. Preliminary results indicate that for certain neuronal populations NIF expression follows, rather than precedes, the terminal mitosis. Further studies on NIF expression in different types of neurones are needed to understand the role of these proteins in development.

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- Holtzer, H. *et al.* in *International Cell Biology*, 1980–1981 (ed. Schweiger, H. G.) 293–305 (Springer, Berlin, 1981).
- Holtzer, H., Fellini, S., Rubinstein, N., Chi, J. & Strahs, K. in *Cell Motility* (eds Goldman, R., Pollard, T. & Rosenbaum, J.) 823–839 (Cold Spring Harbor Laboratory, New York, 1976).
- Goldman, R. D., Milsted, A., Schloss, J. A., Starger, J. & Yerna, M.-J. *A. Rev. Physiol.* **41**, 703–722 (1979).
- Lazarides, E. *Nature* **283**, 249–256 (1980).
- Hoffman, P. N. & Lasek, R. J. *J. Cell Biol.* **66**, 351–366 (1975).
- Liem, R. K. H., Yen, S.-H., Salomon, G. D. & Shelanski, M. L. *J. Cell Biol.* **79**, 637–645 (1978).
- Schlaepfer, W. W. & Freeman, L. A. *J. Cell Biol.* **78**, 653–662 (1978).
- Runge, M. S., Detrich, H. W. III & Williams, R. C. *Jr Biochemistry* **18**, 1689–1697 (1979).
- Czosnek, H., Soifer, D. & Wisniewski, H. M. *J. Cell Biol.* **85**, 726–734 (1980).
- Bennett, G. S., Tapscott, S. J., Kleinbart, F. A., Antin, P. B. & Holtzer, H. *Science* **212**, 567–569 (1981).
- Yen, S.-H. & Fields, K. L. *J. Cell Biol.* **88**, 115–126 (1981).
- Willard, M. & Simon, C. *J. Cell Biol.* **89**, 198–205 (1981).
- Franko, W. W., Schmid, E., Osborn, M. & Weber, K. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5034–5038 (1978).
- Zackroff, R. V. & Goldman, R. D. *Proc. natn. Acad. Sci. U.S.A.* **76**, 6226–6230 (1979).
- Eng, L. F., Vanderhaeghe, J. J., Bignami, A. & Gerstl, B. *Brain Res.* **28**, 351–354 (1971).
- Bignami, A., Eng, L. F., Dahl, D. & Uyeda, C. Y. *Brain Res.* **43**, 429–435 (1973).
- Lazarides, E. & Hubbard, B. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 4344–4348 (1976).
- Small, J. V. & Sobieszek, A. *J. Cell Sci.* **23**, 243–268 (1977).
- Franko, W. W., Weber, K., Osborn, M., Schmid, E. & Freudenstein, C. *Expl Cell Res.* **116**, 429–445 (1978).
- Sun, T. T. & Green, H. *Cell* **14**, 469–476 (1978).
- Tapscott, S. J., Bennett, G. S., Toyama, Y., Kleinbart, F. & Holtzer, H. *Dev Biol.* **85** (in the press).
- Bennett, G. S. *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 4364–4368 (1978).
- Bennett, G. S., Fellini, S. A. & Holtzer, H. *Differentiation* **12**, 71–82 (1978).
- Fellini, S. A., Bennett, G. S., Toyama, Y. & Holtzer, H. *Differentiation* **12**, 59–70 (1978).

- Hamburger, V. & Hamilton, H. L. *J. Morph.* **88**, 49–92 (1951).
- Langman, J. & Haden, C. C. *J. comp. Neurol.* **138**, 419–432 (1970).
- Hollyday, M. & Hamburger, V. *Brain Res.* **132**, 197–208 (1977).
- Jacobson, M. *Developmental Neurobiology* (Plenum, New York, 1978).
- Sechrist, J. W. *Am. J. Anat.* **124**, 117–134 (1969).
- Willigner, M. & Schachner, M. *Dev Biol.* **74**, 101–117 (1980).
- Schmechel, D. E., Brightman, M. W. & Marangos, P. J. *Brain Res.* **190**, 195–214 (1980).
- Rothman, T. P. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 6221–6225 (1980).
- Rothman, T. P., Gershon, M. D. & Holtzer, H. *Dev Biol.* **65**, 322–341 (1978).

A second messenger required for nerve growth factor biological activity?

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After binding to specific membrane receptors of target neurones and responsive pheochromocytoma cells, nerve growth factor (NGF) is internalized and accumulated in the perikaryon within membrane-confined compartments (H. Rohrer *et al.*, unpublished observation, and refs 1–5). Although quantitative electron microscopic autoradiography and ultrahistochemical studies gave no evidence for further movement of NGF^{1,2,4,5}, it was claimed on the basis of light microscopy of PC12 cells (ref. 6 and P.C. Marchisio, personal communication) that some of the NGF taken up by the cell reaches the free cytoplasm and subsequently the nucleus where it would exert a physiological effect. We report here that the direct introduction of NGF into the free cytoplasm of pheochromocytoma cells by fusion with NGF-loaded erythrocyte ghosts does not induce fibre outgrowth, in contrast to the normal response of these cells seen when NGF is added to the culture medium. Correspondingly, NGF antibodies introduced into the cytoplasm do not prevent fibre outgrowth evoked by NGF added to the culture medium. Thus, the binding of NGF to its receptor must result in the production of a second messenger either from the cell surface or after internalization from an intracellular compartment^{7,8}.

Guinea pig erythrocyte ghosts loaded with NGF or NGF antibodies (see Fig. 2 legend) served as vehicles for injecting these molecules into the cytoplasm of NGF target cells through cell fusion. The target cells were a line of PC12 pheochromocytoma cells which reacted rapidly to NGF with an increase in choline acetyltransferase and induction of fibre outgrowth which occurred in 50–70% of single cells within 48 h. In addition to NGF or NGF antibodies, the ghosts were loaded simultaneously with two different marker proteins: fluorescein isothiocyanate-conjugated bovine serum albumin (FITC-BSA), serving as a marker to follow directly the fusion procedure by fluorescence microscopy; and horseradish peroxidase (HRP), to quantify the number of injected cells in fixed preparations. The loading efficiency of the ghosts, that is, the equilibration between the concentration in the loading solution (for details, see Fig. 2 legend) and the interior of the ghosts, depends on the molecular weight (MW) of the protein to be loaded⁹. Accordingly, the 'loading efficiency' for ¹²⁵I-labelled NGF (MW 26,000) was 100%, whereas that for purified anti-NGF IgG antibodies (MW 150,000) was only 30%. The biological activity of NGF or NGF antibodies in the ghosts was tested by their ability either to induce fibre outgrowth or antagonize NGF-induced fibre outgrowth, respectively in PC12 cells. On the average, one loaded ghost contained about 44,000 molecules of NGF or a quantity of NGF antibodies able to neutralize about 26,000 molecules of NGF. Fusion of ghosts with target cells was performed in sparsely seeded monolayer cultures of the latter. In principle, we followed the fusion procedure of Schlegel *et al.*¹⁰ with the modifications specified in Fig. 2 legend. The low temperature (4 °C) used reduced general cell damage during the 5-min exposure to polyethylene glycol (PEG). After fusion, the cells were kept in regular tissue culture conditions at 37 °C. Ghosts which had not fused detached from the cells during the next 6–12 h.

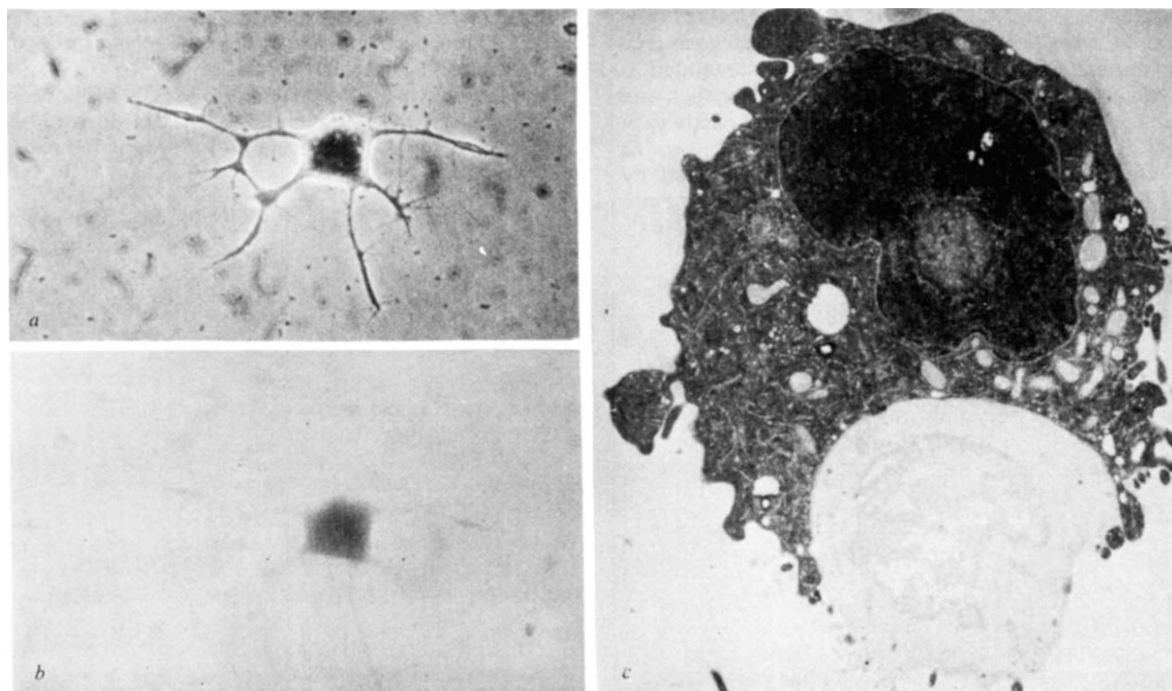
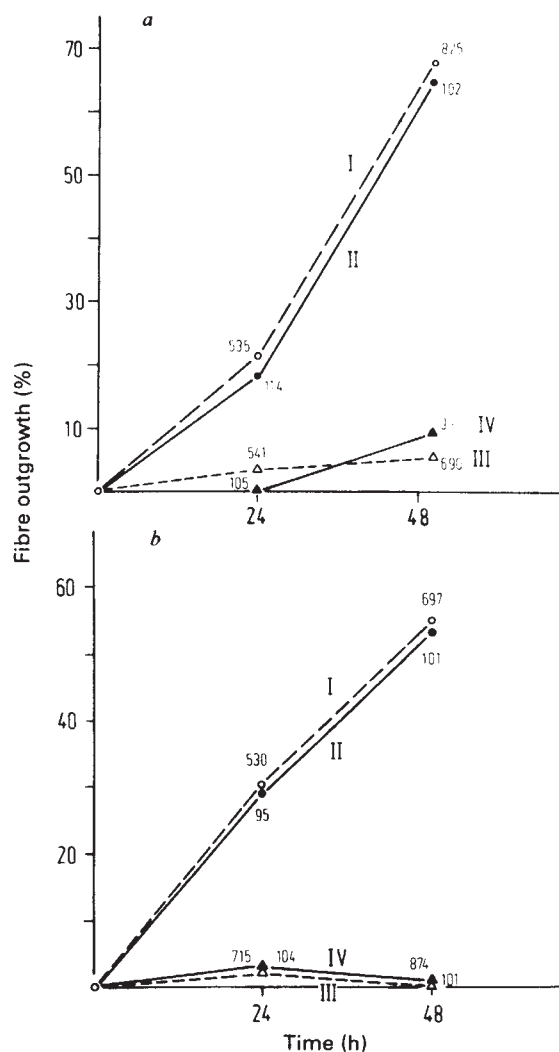


Fig. 1 Phase contrast (a), bright field (b) (same cell) and electron microscopic (c) photographs of fast-responding PC12 cells injected with HRP, FITC-BSA and NGF. The injection procedure is described in detail in Fig. 2 legend. The cells were grown in the presence of 50 ng ml^{-1} NGF. The HRP reaction product¹⁸ is evenly distributed over the whole cell cytoplasm as well as the nucleus. The equal distribution of HRP in the free cytoplasm and the nuclear chromatin becomes impressively apparent in c which depicts neighbouring injected (top) and non-injected (bottom) cells. a, b, $\times 220$; c, $\times 5,055$.

Fig. 2 Effect of the injection of mouse NGF (a) or NGF antibodies (b) into PC12 cells. The loading procedure of the ghosts was essentially that of Yamaizumi *et al.*⁹. a, Guinea pig erythrocytes were equilibrated in 0.5 ml of 'reverse phosphate-buffered saline (PBS)' ('loading solution') containing $10 \mu\text{g}$ NGF, 0.3 mg HRP and 10 mg FITC-BSA (FITC/BSA = 2.4 in terms of molecules). The resulting ghosts were washed four times in Ca/Mg-free PBS (PBS-CMF: 8 g l^{-1} NaCl, 0.4 g l^{-1} KCl, 300 mg l^{-1} KH_2PO_4 , 45 mg l^{-1} $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$, 1 g l^{-1} glucose, 20 g l^{-1} sucrose) and were finally resuspended at 10% (v/v) in the same buffer. The number of NGF molecules per ghost was determined by adding tracer quantities of ^{125}I -NGF (prepared by the lactoperoxidase method¹⁹) to the loading solution. For electron-microscopic autoradiography $296 \mu\text{Ci}$ of ^{125}I -NGF (178×10^3 c.p.m. per ng) was used. The calculation of the loading efficiency was based on an average volume of $90 \mu\text{m}^3$ for guinea pig erythrocytes²⁰. Four days before the fusion experiments 30,000 'fast-reacting PC12' cells were plated on 35-mm poly-ornithine-coated dishes and cells were then incubated in Dulbecco's minimal essential medium (DMEM) containing 10% heat-inactivated horse serum and 5% heat-inactivated fetal calf serum. Cells were washed first with PBS-CMF, then with PBS-CMF containing $130 \mu\text{g ml}^{-1}$ phytohaemagglutinin type V (Sigma), and finally $50 \mu\text{l}$ of ghost suspension (2%, v/v) corresponding to 1.2×10^7 ghosts were added to the cultures. After 4–5 min at 37°C another $25 \mu\text{l}$ (0.6×10^7 ghosts) of the suspension were added and the cultures kept for 3–5 min at 37°C . Plates were then washed carefully with 1 ml PBS-CMF and placed in a refrigerator (4°C) for 2–3 min. Thereafter, $100 \mu\text{l}$ of ice-cold PEG 4000 (Roth, 45% (w/v) in PBS-CMF) was added and cells were left at 4°C for 5 min. PEG treatment was terminated by addition of 1 ml DMEM (containing Ca^{2+} , Mg^{2+}). After 15–45 min at 37°C the medium was changed to normal medium with serum. After 8–12 h (taken as time 0) the medium was changed and cells incubated in the presence of either 50 ng ml^{-1} of NGF (curves I, II) or NGF antibodies (curves III, IV). The amount of NGF antibodies used was sufficient to neutralize all the NGF added previously to the cultures in loaded ghosts. At the times indicated cultures were fixed (2.5% glutaraldehyde, 20 min) and stained for HRP (30 min 0.03% diaminobenzidine, 2 h 0.03% diaminobenzidine plus 0.01% H_2O_2)¹⁸. Randomly chosen regions of the plate were scored for fibre outgrowth (positive score for fibres $\geq 20 \mu\text{m}$). The percentage of all the single cells carrying fibres was counted under phase contrast (curves I, III). Subsequently, the injected HRP-positive cells were evaluated under bright-field optics (curves II, IV). The number of cells counted per point are indicated and the injection frequencies can be directly derived from them. b, The same methods as described for a were used with the following modifications. Erythrocytes were loaded in $720 \mu\text{l}$ of a solution containing $504 \mu\text{g}$ affinity-purified NGF antibodies²¹ (neutralizing $40 \mu\text{g}$ NGF), 0.4 mg HRP and 10 mg FITC-BSA. At time 0 those plates which were subsequently incubated with 50 ng ml^{-1} of NGF (curves I, II) were washed with NGF-containing medium. Residual NGF antibody activity could be neutralized by this procedure. The rest of the plates received normal medium (curves III, IV). Injected cells (curves II, IV) and total cells (curves I, III) were evaluated as described for a.



The quantity of ^{125}I -NGF present in the injected cells revealed that an average of six ghosts had fused with each cell. This high multiplicity is probably because the ghosts tended to aggregate with each other and so fusion took place between one PC12 cell and an aggregate of fused ghosts. The radioactivity in ^{125}I -NGF-injected cells decreased gradually over 2 days to ~30% of the initial value. About 50% of the radioactivity remaining after 2 days represented native NGF, as judged from its migration in SDS-polyacrylamide gel electrophoresis using a 15% gel. Thus, the quantity of native NGF present in a single injected cell amounts to an average of ~44,000 molecules 2 days after the injection. In comparison, when PC12 cells are incubated for 24 h with 100 ng ml^{-1} of ^{125}I -NGF, the quantity of radioactivity accumulated (not corrected for possible degradation products) corresponds to ~15,000 molecules per cell—one-third of the amount of NGF injected. As shown in Fig. 1, the injected HRP is evenly distributed throughout the cytoplasm and nucleus of the cell. Electron microscopic autoradiography performed on the same sections indicated that the ^{125}I -labelled NGF, too, was distributed throughout the cell cytoplasm and also reached the nucleus.

The fusion procedure did not influence the NGF-mediated fibre outgrowth, that is, the frequency and extent of fibre outgrowth from injected cells in response to NGF added to the medium is the same as that of non-injected cells (Fig. 2). In those experiments where the response of the NGF-injected cells (in the absence of NGF in the culture medium) was studied, we supplied the medium with NGF antibodies to ensure that any NGF originating from leaking ghosts would not reach cell-surface NGF receptors. As demonstrated in Fig. 2a, the NGF-injected and non-injected cells behaved identically: fibre outgrowth occurred only if NGF was added to the culture medium. Conversely, if NGF antibodies were injected into the cytoplasm these antibodies did not interfere with fibre outgrowth produced by NGF in the culture medium (Fig. 2b). Preliminary results indicate that injected NGF is also unable to support the survival of neurones dissociated from 8-day old embryonic chick dorsal root ganglia or PC12 cells in serum-free medium (in serum-free medium PC12 cells need NGF for survival¹¹), whereas injected anti-NGF antibodies do not antagonize the ability of NGF in the culture medium to support the survival of these cells.

We conclude that even if an undetectably small proportion of NGF leaves the membrane-bound compartments in which it is always localized after receptor-mediated internalization, it would be unable to initiate the characteristic cellular responses to NGF—fibre outgrowth and cell survival. Thus the enhancement of polymerization of microtubules and microfilaments, demonstrated in the test tube in response to high concentrations of NGF^{12–14}, must have no physiological significance in the context of fibre outgrowth, and the binding of NGF to chromatin isolated from chick dorsal root ganglia¹⁵ cannot be responsible for fibre outgrowth or for survival. The biological effects of NGF must be mediated by an interaction with specific cell-surface receptors and the generation of (a) second messenger(s) rather than by a direct action of intact NGF in the free cytoplasm or the nucleus.

The nature of the second messenger and its site of production (cell surface or cell interior after internalization) are unknown. In adrenergic neurones NGF binds to receptors or nerve terminals and is subsequently transported within vesicles and cisternae to the cell body. There, fusion of the transport vesicles with other intracellular membrane systems and eventually with lysosomes can be observed^{2,5}. It cannot be excluded that a cleavage product of NGF is transferred into the cytoplasm as is the case for diphtheria toxin where a fragment of the original toxin molecule is transferred across the membrane into the free cytoplasm where it exerts its blocking action on ribosomal protein synthesis^{16,17}. It is also possible that the NGF-receptor complex is transferred into the cytoplasm, although no such mechanism involving a polypeptide hormone and its receptor has previously been described. If NGF should be cleaved or bound to its receptor before transfer into the cytoplasm, then

our results show that the resulting secondary product(s) of NGF or NGF-receptor complexes are not recognized by the antibodies raised against native NGF.

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- Schwab, M. & Thoenen, H. *Brain Res.* **122**, 459–474 (1977).
- Schwab, M. *Brain Res.* **130**, 190–196 (1977).
- Levi, A., Schechter, Y., Neufeld, E. J. & Schlessinger, J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 3469–3473 (1980).
- Claude, Ph., Dunis, D. A. & Hawrot, E. *J. Cell Biol.* **83**, 632 (1979).
- Schwab, M., Suda, K. & Thoenen, H. *J. Cell Biol.* **72**, 798–810 (1979).
- Marchisio, P. C., Naldini, L. & Calissano, P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1656–1660 (1980).
- Thoenen, H. & Barde, Y.-A. *Physiol. Rev.* **60**, 1284–1335 (1980).
- Thoenen, H., Schäfer, Th., Heumann, R. & Schwab, M. in *Hormones and Cell Regulation* Vol. 5 (eds Dumont, J. E. & Nunez, J.) 15–34 (Elsevier, Amsterdam, 1981).
- Yamaizumi, M., Uchida, T., Mekada, E. & Okada, Y. *Cell* **18**, 1009–1014 (1979).
- Schlegel, R. A. & Mercer, W. E. in *Introduction of Macromolecules into Viable Mammalian Cells* (eds Baserga, R., Croce, C. & Rovera, G.) 145–155 (Liss, New York, 1980).
- Greene, L. A. *J. Cell Biol.* **78**, 747–755 (1978).
- Calissano, P. & Cozzari, C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 2131–2135 (1974).
- Levi, A., Cimino, M., Mercanti, D., Chen, J. S. & Calissano, P. *Biochim. biophys. Acta* **399**, 50–60 (1975).
- Calissano, P., Monaco, G., Castellani, L., Mercanti, D. & Levi, A. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2210–2214 (1978).
- Andres, R. Y., Jeng, J. & Bradshaw, R. A. *Proc. natn. Acad. Sci. U.S.A.* **74**, 2785–2789 (1977).
- Pappenheimer, A. M. Jr & Gill, D. M. *Science* **182**, 353–358 (1973).
- Collier, J. R. *Bact. Rev.* **39**, 54–85 (1975).
- Mazurkiewicz, J. E. & Nakane, P. K. *J. Histochem. Cytochem.* **20**, 969–974 (1972).
- Sutter, A., Riopelle, R. J., Harris-Warrick, R. M. & Shooter, E. M. *J. biol. Chem.* **254**, 5972–5982 (1979).
- Valet, G., Hofmann, H. & Ruhenstroth-Bauer, G. *J. Histochem. Cytochem.* **24**, 231–246 (1976).
- Stockel, K., Gagnon, C., Guroff, G. & Thoenen, H. *J. Neurochem.* **26**, 1207–1211 (1976).

Reversal of transplantation immunity by liver grafting

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The transplantation of organs between individuals of a species normally has two main consequences: (1) the tissue is rejected unless the individuals are matched for identity of transplantation antigens, especially those encoded by the major histocompatibility complex (MHC), and (2) the recipient is sensitized to the transplantation antigens of the donor so that second-set grafts are rejected in a more violent manner¹. These rules of transplantation are not obeyed by liver grafts. Orthotopic transplants of liver are never rejected by many strains of rat even though the MHC barrier is crossed². We have recently shown that instead of sensitizing the recipient, these enduring liver grafts induce a state of donor-specific unresponsiveness in which subsequent grafts of other organs, such as skin, are accepted permanently. It is possible that many strains simply cannot mount a sufficiently vigorous destructive immune response to outstrip the liver's great capacity to repair immune damage and that the systemic unresponsiveness observed is due to diversion of alloreactive lymphocytes with donor specificity into the liver allograft. Manipulations which increase the vigour of the immune response might therefore tip the balance in favour of liver graft rejection and away from induction of unresponsiveness. We have previously measured the degree to which the immune response can be increased by previous exposure to MHC antigens in a quantitative adoptive transfer system³. In the strain combination DA (MHC haplotype RT-1^a) grafted to PVG (RT-1^k), the lymphocytes of immunized animals are 1,000 times more potent than those of non-immune animals in procuring graft destruction when transferred to irradiated hosts. Unexpectedly, we have now found that liver grafts transplanted into previously immunized rats not only fail to reject but convert the state of heightened reactivity to donor grafts characteristic of immune recipients into one of non-reactivity characteristic of tolerant animals.

Table 1 Induction of tolerance to MHC antigens by liver transplantation is systemic

Days after DA liver grafts	Days of skin graft survival (no. of rats)	
	DA	AO
0	10, 12, 13, 14(2), 18	8, 9, 12
5	13, 14, 15, 22, 100 (2)	11, 12 (2)
15	23* > 100 (5)	13 (2), 14
45	65*, 88*, >100 (4)	13 (2), 15

PVG rats received an orthotopic transplant of liver from DA donors on day 0 and then an orthotopic skin graft from both DA and AO (third party) donors on the day indicated. By day 15 some recipients were systemically tolerant of the DA alloantigens, as indicated by permanent survival of the indicator DA skin graft in perfect cosmetic condition. By day 45 all animals were tolerant. The specificity of this tolerance was absolute, as indicated by rejection of all third party (AO, RT1^a) skin grafts.

* These animals died with a perfect DA skin graft in place.

The technique for orthotopic liver transplantation used in these experiments is reported in detail elsewhere⁴. Surgical technique is not a significant variable in this series, as indicated by a 95% permanent survival rate of the past 72 DA to PVG liver transplants performed here (Fig. 1). Although DA livers transplanted to PVG recipients are never rejected, skin grafts are rejected in 8 days, heart grafts in 7–8 days and kidney grafts in 9–10 days. The reason for the prolonged survival of the liver in the rat and in other species⁵ is still being studied in this and in other laboratories, but it is not due to a failure of the recipients to mount an immune response against liver tissue. Indeed, the degree of early mononuclear cell infiltration, blast transformation and liver cell necrosis, as assessed histologically, is almost as great in PVG recipients which do not reject as in BN recipients which do (N.K., Wight and B.R., in preparation). The response in PVG rats is ephemeral, only minimal histological changes being detectable several months after grafting, whereas the response in BN rats is progressive.

While grafting with skin, heart or kidney provokes vigorous immunity, grafting with liver rapidly induces a state of systemic unresponsiveness (Table 1). Thus, within 15 days of liver transplantation, recipient rats become specifically unresponsive to subsequent skin grafts from the DA strain while retaining the capacity to reject third party (AO, RT1^a) strain grafts. This state of unresponsiveness is associated with the presence of specific blocking material in the circulation⁶ and deletion of the clones of cells required for DA graft rejection from the peripheral lymphocyte pool, but there is no evidence of cell-mediated suppression (data not shown).

Because we had previously established that immunization to antigens of the MHC in this strain combination led to a large

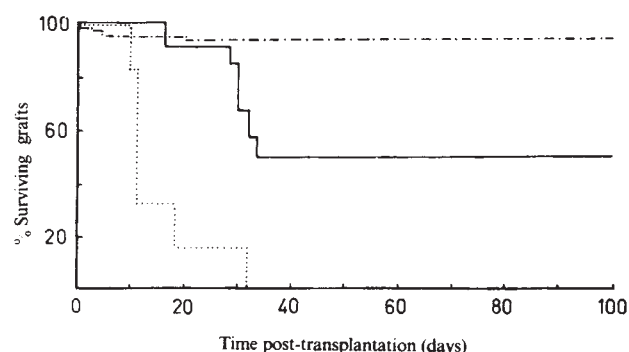


Fig. 1 Survival of liver grafted rats. Apart from three early technical failures⁵, 69 normal PVG rats grafted with DA livers (—) survived indefinitely while BN rats grafted with DA livers (·····) all died within 32 days. When PVG rats were sensitized by rejection of DA skin grafts 28 days before liver transplantation (---), 50% behaved as high responder animals, rejecting their liver grafts within 34 days, while 50% failed to reject and survived indefinitely.

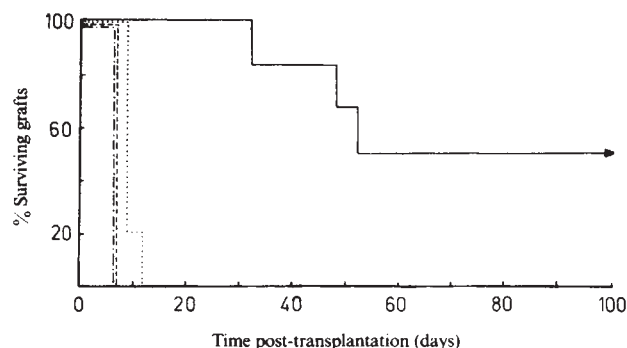


Fig. 2 Immune status of liver grafted rats. PVG rats, immunized to DA antigens, which had survived subsequent grafting with DA livers, were challenged with another DA skin graft 28 days after liver transplantation (—). All rats showed very prolonged survival of the second-set DA skin graft and 50% of animals retained the skin graft indefinitely. Where no liver graft intervened between the two DA skin grafts (·····) or where the liver graft was syngeneic with the recipient PVG (---), the second skin graft was rapidly rejected in second-set tempo. Substitution of third party (AO) antigens at the immunizing and challenge stages (---) showed that the DA liver graft was not nonspecifically immunosuppressive: the second AO skin grafts, placed after DA liver transplantation on PVG rats immunized by prior skin grafts, were all promptly rejected.

increase in the efficiency of heart graft rejection as measured in a quantitative adoptive transfer assay³, we felt confident that immunization would convert the non-rejector PVG to a rejector strain of equivalent potency to BN. Surprisingly, 50% of 12 previously immunized animals survived indefinitely after subsequent DA liver transplantation (Fig. 1). In those six animals which died after liver grafting, histological examination revealed a severe destructive immune response characterized by heavy mononuclear cell infiltrates and widespread hepatocellular necrosis. In the six animals which survived long-term, the livers showed minimal signs of tissue destruction and no significant cellular infiltrate (a picture similar to that seen long-term in normal rats in receipt of DA liver grafts). It was therefore of interest to determine whether, like normal non-immune graft recipients, the immune animals had also become systemically tolerant to DA MHC antigens during the recovery phase. This proved to be the case. Subsequent skin grafts applied to such animals failed to reject in 50% of cases, for over 100 days (Fig. 2). The antigen specificity of this phenomenon was shown in two ways. One group of immunized animals received a syngeneic (PVG) liver graft instead of a DA graft. This has no suppressive effect on the second-set rejection of DA skin (Fig. 2). Another group of PVG rats was immunized against third party AO (RT1^a) antigens instead of DA (RT1^a) antigens before receiving a DA liver graft. In this group the fully allogeneic DA liver graft did not induce cross-tolerance to AO antigens, as indicated by rejection of all subsequent AO indicator skin grafts.

Using easily enhanced rat strains, Bowen *et al.*⁷ demonstrated a slight weakening of immunity against F₁ hybrid kidney grafts by temporary parabiosis of hyperimmune animals to animals bearing enhanced kidney grafts. Kawamura *et al.*⁸, again using F₁ hybrid kidney grafts, demonstrated that second-set grafts of identical type often have prolonged survival especially if the recipient animal was unable to reject the first graft promptly. However, they could only demonstrate this phenomenon where exposure to the first graft had not led to detectable sensitization of the recipient⁹. We believe this to be the first observation of specific conversion of a state of transplantation immunity against a full haplotype MHC difference to a state of tolerance. It had previously proved impossible both clinically and experimentally to erase memory of previous exposure to antigens of the MHC even with such potent immunosuppressive agents as cyclosporin A¹⁰. The facility with which liver transplantation achieves this result in a high proportion of animals undermines

the simple quantitative explanation of liver graft survival outlined in the introduction and obviously deserves further study. Of particular interest will be the analysis of the efficacy of liver tissue in the form of an intact organ or as dispersed cells in modifying the alloreactive repertoire of normal and immune animals.

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1. Billingham, R. E., Brent, L. & Medawar, P. B. *Proc. R. Soc. B* **143**, 58–60 (1954).
2. Kamada, N., Brons, G. & Davies, H. F. S. *Transplantation* **29**, 429–431 (1980).
3. Hall, B. M., Dorsch, S. E. & Roser, B. *Transplantation* **26**, 357–359 (1978); *J. exp. Med.* **148**, 878–890, 891–903 (1978).
4. Kamada, N. & Calne, R. Y. *Transplantation* **28**, 47–50 (1979).
5. Calne, R. Y. *et al. Nature* **233**, 472–476 (1969).
6. Kamada, N., Davies, H. F. S. & Roser, B. *Transplant. Proc.* **13**, 837–841 (1981).
7. Bowen, J. E. *et al. Transplantation* **18**, 322–327 (1974).
8. Kawamura, H., Mullen, Y. & Hildermann, W. H. *Transplantation* **30**, 302–307 (1980).
9. Kawamura, H. *et al. Transplant. Proc.* **13**, 114–116 (1981).
10. Homan, W. P. *et al. Transplantation* **29**, 361–366 (1980).

Immune (γ) interferon produced by a human T-lymphoblast cell line

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Interferons (IFNs) are glycoproteins with antiviral, immunomodulatory and antiproliferative properties. They are classified on the basis of their acid stability, antigenic properties, stimuli for production and cellular origin. IFN- α and IFN- β are acid-stable and derived from leukocytes and fibroblasts. IFN- γ (immune, type II) is acid-labile and generally derived from T lymphocytes. Elaboration of IFN- γ occurs either after sensitized T lymphocytes are exposed to specific antigen or on induction of T lymphocytes with mitogens^{1,2}. IFN- α and IFN- β have been extensively characterized and much is known about their biochemical properties and gene structure^{3–11}. In contrast, there is relatively little information available regarding the physical and biological properties of human IFN- γ (refs 1, 2, 12–17). Particular interest in IFN- γ derives from *in vitro* and *in vivo* studies which indicate that IFN- γ has greater antiproliferative effects on neoplastic cells than IFN- α and IFN- β (refs 18–21). We report here the production and characteristics of IFN- γ elaborated by a unique human T-lymphoblast cell line.

The paucity of data regarding IFN- γ is largely related to difficulties in obtaining large quantities of purified material. In our laboratory, a human T-lymphocyte cell line (Mo) has been derived from the spleen of a patient with a T-cell variant of hairy-cell leukaemia that produces IFN- γ and other lymphokines. The Mo cell line has been described in detail elsewhere^{22–26}.

The Mo cells constitutively produce low levels of interferon, both in the presence and absence of serum, and its production is not enhanced by exposing the cells to Sendai virus or polyinosinic polycytidylic acid. We tried to induce IFN- γ production using T-cell mitogens as well as chemical agents (Table 1). Induction with phytohaemagglutinin (PHA) markedly increased the production of IFN- γ . As the ranges in Table 1 demonstrate, there was considerable variability in the titres elaborated in each induction regimen. Interferon production by Mo cells in culture was maximal after 3 to 4 days of incubation with PHA, thus the conditioned media used in all experiments were collected after 4 days of induction.

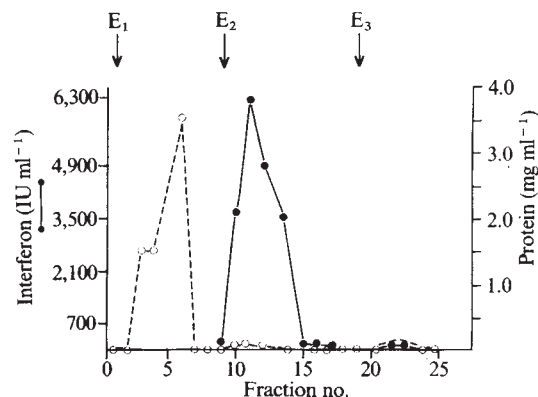


Fig. 1 Chromatography of Mo IFN- γ on Con A-Sepharose. Conditioned medium containing 2% FCS induced with 3% PHA, 1 μ M dexamethasone and 0.5 mM sodium butyrate was dialysed for 16 h at 4°C against 100 volumes of PBS, pH 7.4 (E₁). The Con A-Sepharose (Pharmacia) column (0.9 × 5 cm) was equilibrated with PBS, and 5 ml of dialysed conditioned medium applied. The column was washed with PBS then developed with 0.2 M α -D-methyl mannoside in PBS (E₂) at a flow rate of 2 ml h⁻¹. The column was developed with 0.2 M α -D-methyl mannoside and 20% ethylene glycol in PBS (E₃). Protein was determined using a dye binding procedure (Bio-Rad). Solid line represents interferon activity and broken line, protein concentration.

It has been shown^{15,17,27,28} that chemical agents known to affect cell differentiation *in vitro* may enhance IFN production. The phorbol ester, 12-O-tetradecanoylphorbol-13-acetate (TPA) proved to be a potent enhancing agent of IFN- γ production by the Mo cells. Levels of up to 43,400 international units (IU) per ml per 10⁶ cells were achieved in the presence of 2% fetal calf serum (FCS), 3% PHA and TPA (10 ng ml⁻¹). However in serum free conditions, PHA induction alone gave maximal interferon yield with specific activities of $\sim 2 \times 10^4$ IU per mg protein. Concanavalin A (Con A; 20–40 μ g ml⁻¹) also induced IFN- γ production but its effect was substantially less than that observed for PHA (data not shown).

Mo interferon proved to be entirely of the γ type by the criteria of acid lability, stimulus of induction, kinetics of production and failure of neutralization by antisera to leukocyte interferon (data not shown). The antiviral activity in Mo-conditioned medium was totally inactivated on reduction to pH 2 with 0.1 M HCl or 0.5 M citrate buffer. Also, Mo IFN- γ is heat-labile, losing >98% activity when incubated at 56°C for 1 h or on boiling for 1 min (Table 2). This observation is consistent with previous reports which indicate that mitogen-induced IFN- γ is heat-labile^{12,14}. We examined the effect of various denaturing agents on the IFN- γ and found that it was almost completely inactivated with 8 M urea, 6 M guanidine hydrochloride, 0.1% SDS and 0.01% zwitterionic detergent. However, note that there was retention of activity after incubation with 10 mM β -mercaptoethanol (Table 2), suggesting that, while disulphide linkages may be present in the molecule, they are not critical for antiviral activity. The activity of Mo IFN- γ is completely destroyed by pronase (Table 2).

The chromatographic behaviour of Mo IFN- γ on Con A-Sepharose is shown in Fig. 1. The bulk of the protein applied to the column passed through unretarded and this material contained only negligible amounts of interferon activity. The bound interferon was recovered by elution with 0.2 M α -D-methyl mannoside in phosphate-buffered saline (PBS; pH 7.4) giving a purification of ~ 20 -fold to a specific activity of 6 – 8×10^4 IU per mg protein; 96% of the interferon activity loaded was recovered in the α -D-methyl mannoside eluate. Only 0.5% of the applied activity was recovered after elution with 20% ethylene glycol, 0.2 M α -D-methyl mannoside in PBS. These results suggest that almost all the Mo IFN- γ is glycosylated. It has previously been reported²⁹ that human IFN- γ induced in

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Table 1 Induction of IFN- γ from Mo cells

	IFN titre (IV per ml per 10^6 cells)	
	Mean	Range
Serum-free medium		
Uninduced	35	5–50
PHA	1,900	200–3,200
PHA + TPA (10 ng ml $^{-1}$)	1,100	800–1,200
Medium + serum (2% FCS)		
Uninduced	66	50–75
PHA	5,700	900–9,400
PHA + dexamethasone (1 μ M)	4,350	2,700–6,000
PHA + butyrate (0.5 mM)	4,100	900–7,900
PHA + butyrate (0.5 mM) + dexamethasone (1 μ M)	5,150	3,500–7,900
PHA + TPA (10 ng ml $^{-1}$)	16,900	7,871–43,400

The Mo cells were cultured in α medium (Flow) containing 20% FCS (screened lot). Interferon production, unless otherwise stated, was achieved by washing the cells three times with α medium and then culturing 10^6 cells ml $^{-1}$ for 4 days in α medium alone (serum-free) or in α medium containing 2% FCS. Inducers used were: phytohaemagglutinin (PHA; HA 15 reagent grade, Wellcome), sodium butyrate (Pfaltz & Bauer), dexamethasone (Sigma) and TPA (PL Biochemicals); these were added to the culture at the concentrations shown. PHA was present at 2% and 3% in serum-free and serum-containing cultures, respectively. The antiviral activity of interferon was determined by quantitation of the cytopathic effect of murine encephalomyocarditis virus on the foreskin fibroblast cell line CCL54 trisomic for chromosome 21 (ref. 30). All interferon units are expressed with reference to the NIH human leukocyte interferon standard (G-023-901-527). Titres are the results of four separate experiments.

leukocytes by PHA shows chromatographic heterogeneity on Con A-Sepharose, presumably resulting from varying degrees of glycosylation. In contrast, the Mo IFN- γ appears more homogeneous with respect to its glycosylation.

The hydrophobic nature of human IFN- γ has been described in previous studies^{12–14}. Crude Mo-conditioned medium, induced in the presence of 2% FCS with 3% PHA, 0.5 mM butyrate and 1 μ M dexamethasone, was applied to a controlled-pore glass (CPG) 350-B mesh size 120/200 column (Electronucleonics). All the interferon activity was absorbed onto the column while 98% of the protein was voided. Elution with 1 M NaCl, 20% ethylene glycol in 0.2 M phosphate buffer (pH 7.4) recovered 2% of the loaded protein and almost all the interferon activity, giving a ~ 50 -fold purification and a specific activity of $\sim 10^5$ IU per mg protein.

Reported molecular weights (MWs) for IFN- γ have ranged from 20,000 to 80,000—these discrepancies may be related to molecular heterogeneity and binding to other proteins. Gel-

Table 2 Characterization of Mo IFN- γ

Treatment	Residual interferon activity (% of control)
Urea (8 M)	4
Guanidine HCl (6 M)	0.7
β -Mercaptoethanol (10 mM)	100
SDS (0.1%)	0
Zwitterionic detergent (0.01%)	9
56 °C (1 h)	1.6
100 °C (1 min)	1.8
Pronase (0.1 mg ml $^{-1}$)	<0.1
pH 2	0

Conditioned medium containing 2% FCS obtained from Mo cells cultured with 3% PHA and 10 ng ml $^{-1}$ TPA was used. All treatments except heat and pronase inactivation were performed at 37 °C for 1 h. The material was then dialysed against 100 volumes of PBS, with two exchanges. Pronase (Sigma) was incubated with serum-free conditioned medium from cells induced with 2% PHA alone at 37 °C for 1 h. Control incubations with 2% FCS contained 2,000 IU ml $^{-1}$ interferon activity whereas the serum-free material had 3,200 IU ml $^{-1}$.

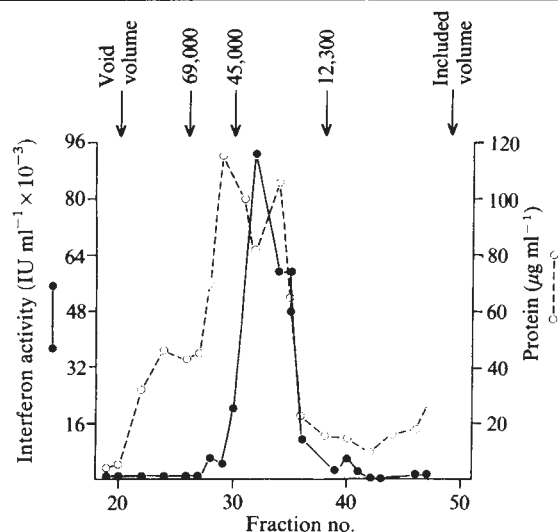


Fig. 2 Gel filtration of Mo IFN- γ . Serum-containing conditioned medium from Mo cells induced with 3% PHA and TPA (10 ng ml $^{-1}$) was partially purified on CPG beads and then applied to an Ultrogel AcA 44 (LKB) column (0.9 $\times$ 40 cm) equilibrated with 20% ethylene glycol, 1 M NaCl in 0.02 M phosphate buffer, pH 7.4. Column was developed with the same buffer at a flow rate of 2.2 ml h $^{-1}$. Fractions of 0.75 ml were collected. Molecular weight standards were: blue dextran (void volume), bovine serum albumin (MW 69,000), ovalbumin (MW 45,000), cytochrome c (MW 12,300) and 4-methylumbelliferone (included volume). Solid line represents interferon activity, and broken line, protein concentration.

filtration chromatography of serum-containing conditioned medium was performed using an Ultrogel AcA 44 column. An initial peak of interferon was obtained with the PBS elution at $\sim 70,000$ MW. The specific activity of this material was $\sim 10^4$ IU per mg protein. Two smaller peaks of interferon activity were obtained with 1 M NaCl elution and then with 1 M NaCl, 20% ethylene glycol. The secondary peaks may represent species of interferon exhibiting ionic or hydrophobic interaction with the Ultrogel beads. To examine further the size characteristics of the Mo IFN- γ , we applied material partially purified on CPG beads to a similar Ultrogel AcA 44 column (Fig. 2). To avoid ionic or hydrophobic interactions and focus solely on its gel-filtration properties, the column was developed using 1 M NaCl, 20% ethylene glycol in 0.02 M phosphate buffer (pH 7.4). A single peak of activity was obtained with an apparent molecular weight of $\sim 40,000$ and a specific activity of 1.2×10^6 IU per mg protein. It is possible that the high-molecular weight species observed when crude conditioned medium was applied directly to the gel-filtration column represented binding of Mo IFN- γ to carrier proteins present in serum.

Mo IFN- γ is a hydrophobic glycoprotein which is elaborated in high titre on induction with T-cell mitogens and various chemical agents. It is clear from its physicochemical characteristics that the Mo IFN- γ is distinct from the erythroid-potentiating activity, colony-stimulating activity and neutrophil migration-inhibitory factor also produced by this cell line^{22–26}. As the Mo cell line is a homogeneous population of T lymphoblasts, the results suggest that IFN- γ may be induced by lectins without the need for cooperation by other cell types².

The Mo cell line provides a unique source of human IFN- γ , and should allow the large-scale production of IFN- γ for purification and clinical trials. It may also be of use in isolating the human gene(s) for IFN- γ .

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- Stewart II, W. E. *The Interferon System* (Springer, Vienna, 1979).
- Epstein, L. B. in *Biology of the Lymphokines* (eds Cohen, S. & Oppenheim, J. J.) 443–514 (Academic, New York, 1979).
- Rubinstein, M. et al. *Proc. natn. Acad. Sci. U.S.A.* **76**, 640–644 (1979).
- Zoon, K. C. et al. *Science* **207**, 527–528 (1980).
- Knight, E. Jr, Hunkapiller, M. W., Korant, B. D., Hardy, R. W. F. & Hood, L. E. *Science* **207**, 525–526 (1980).
- Derynck, R. et al. *Nature* **285**, 542–546 (1980).
- Houghton, M. et al. *Nucleic Acids Res.* **8**, 1913–1931 (1980).
- Nagata, S., Mantei, N. & Weissmann, C. *Nature* **287**, 401–408 (1980).
- Goeddel, D. V. et al. *Nature* **290**, 20–26 (1981).
- Taniguchi, T. et al. *Nature* **285**, 547–550 (1980).
- Allen, G. & Fantes, K. H. *Nature* **287**, 408–411 (1980).
- Langford, M. P., Georgiades, J. A., Stanton, G. J., Dianzani, F. & Johnson, H. M. *Infect. Immun.* **26**, 36–41 (1979).
- Wiranowska-Stewart, M., Lin, L. S., Braude, I. A. & Stewart, W. E. II *Molec. Immun.* **17**, 625–633 (1980).
- de Ley, M. et al. *Eur. J. Immun.* **10**, 877–883 (1980).
- Vilček, J., Sulea, I. T., Volvovitz, F. & Yip, Y. K. in *Biochemical Characterization of Lymphokines* (ed. de Weck, A. L.) 323–329 (Academic, New York, 1980).
- Marcucci, F., Waller, M., Kirchner, H. & Krammer, P. *Nature* **291**, 79–81 (1981).
- Yip, Y. K., Pang, R. H. L., Urban, C. & Vilček, J. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1601–1605 (1981).
- Bloom, B. R. *Nature* **284**, 593–596 (1980).
- Krim, M. *Blood* **55**, 875–884 (1980).
- Rubin, B. Y. & Gupta, S. L. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5928–5932 (1980).
- Glasgow, L. A., Crane, J. L. Jr & Kern, E. R. *J. natn. Cancer Inst.* **60**, 659–666 (1978).
- Golde, D. W., Quan, S. G. & Cline, M. J. *Blood* **52**, 1068–1072 (1978).
- Saxon, A., Stevens, R. H. & Golde, D. W. *Ann. intern. Med.* **88**, 323–326 (1978).
- Golde, D. W., Bersch, N., Quan, S. G. & Lusis, A. J. *Proc. natn. Acad. Sci. U.S.A.* **77**, 593–596 (1980).
- Lusis, A. J., Quon, D. H. & Golde, D. W. *Blood* **57**, 13–21 (1981).
- Weisbart, R. H., Golde, D. W., Spolter, L., Eggena, P. & Rinderknecht, H. *Clin. Immun. Immunopath.* **14**, 441–448 (1979).
- Adolf, G. R. & Swetly, P. in *In Vivo and In Vitro Erythropoiesis: The Friend System* (ed. Rossi, G. B.) 577–584 (Elsevier, Amsterdam, 1980).
- Adolf, G. R. & Swetly, P. *Nature* **282**, 736–738 (1979).
- Mizrahi, A. et al. *J. biol. Chem.* **253**, 7612–7615 (1978).
- Bryson, Y. J. & Kronenberg, L. H. *Antimicrob. Ag. Chemother.* **11**, 299–306 (1977).

Establishment of a human T-cell hybrid line with suppressive activity

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The construction of murine B hybridomas producing homogeneous specific antibodies in almost unlimited quantities has led to major progress in immunology¹. Recently, human B hybrid lines secreting antibody of defined specificities have also been established^{2,3}. Similarly, murine T hybrids have been obtained which may produce specific immunoregulatory factors such as antigen-specific suppressor factors^{4–6}, T-cell replacing factor⁷ or allogeneic effect factor⁸. The availability of functional T hybrids in man should allow a better understanding of human lymphocyte interaction and regulation. We describe here the fusion of a permanent human T-cell (KE37) with peripheral blood T lymphocytes to produce a human T hybridoma line which has a stable hypotetraploid karyotype and continuously produces a factor which suppresses pokeweed mitogen (PWM)-induced B-cell differentiation.

The permanent T-cell line used for hybridization was KE37, which has been derived from a human acute lymphocytic leukaemia (provided by H. G. Kunkel and S. M. Fu). A 5-bromodeoxyuridine (BUdR)-resistant variant of this line was selected after mutagenesis to obtain a line deficient in thymidine kinase and therefore sensitive to the hypoxanthine-aminopterin-thymidine (HAT) medium⁹. This variant line was then subcloned by limiting dilution. We fused one of these clones, D1R11, with peripheral blood lymphocytes (PBL) from a patient who presented with a variable agammaglobulinaemia and a moderate blood T-cell hyperlymphocytosis (5,000 mm⁻³); most of these T lymphocytes expressed Ia antigen and showed a

Table 1 Cell-surface phenotype of parental and hybrid cells (expressed as percentage of positive cells)

	Surface	Ig	E rosettes	T3	T4	T5	T6	T8	T10	T11	Ia
DE	0	20	73	15	50	0	50	NT	NT	60	
D1R11	0	10	0	0	0	100	10	50	4	0	
DE×D1R11	0	70	0	0	0	100	100	100	100	0	

10×10⁶ cells from a subclone (D1R11) of the thymidine kinase-deficient variant of KE37 T-cell line were fused with 10×10⁶ peripheral T cells from donor DE. For the fusion the parental cells were washed twice with Dulbecco's minimal essential medium (DMEM) without fetal calf serum (FCS), the last washing being performed in the presence of 5% dimethyl sulphoxide (DMSO). The pellet was then resuspended in 0.5 ml of 33% (w/v) polyethylene glycol (PEG) (molecular weight (MW) 4,000; Sigma), left for 3 h at 37°C then centrifuged for 3 h at 200g. The supernatant was carefully discarded, the cells gently resuspended in DMEM plus 20% FCS then placed in 24-well Costar microtitre plates at a concentration of 1×10⁶ cells per well, with a feeder of rat fibroblasts (0.1×10⁶ cells per well). Selective HAT medium (aminopterin, 4×10⁻⁷M; thymidine, 1.6×10⁻⁵M; hypoxanthine, 1.1×10⁻⁴M) was added on days 2, 4, 8 and 10 after fusion, then replaced by HT medium for 6 days. Surface markers were studied on parental cells and on a given hybrid clone (DE×D1R11). Surface immunoglobulin (Ig) was detected with rhodamine-conjugated F(ab)₂ fragments of rabbit IgG antibody to human immunoglobulin. Monoclonal antibodies to Ia and T-cell antigens (designated T3, T4, T5, T6, T8 and T10) were detected by immunofluorescence using a second layer of fluorescein-conjugated IgG from a goat anti-mouse immunoglobulin serum (Nordic).

suppressor/cytotoxic antigenic phenotype (Table 1) as defined by the series of monoclonal OKT antibodies developed by Reinherz and Schlossman (reviewed in ref. 10; provided by G. Goldstein from Ortho Research Laboratory).

The fusion protocol used is described in Table 1 legend. In this, as well as in other experiments which used a variety of normal or stimulated T cells fused with either KE37 subclones or two other T-cell lines (JM and CEM), we found that the yield of hybrid clones was low; in this fusion 1 well out of 24 contained hybrids and their growth was delayed by 3–5 weeks. Subcloning the parental KE37 variant possibly improved the efficiency of fusion but it seems that human T-cell hybrids require special culture conditions during their initial growth phase. The hybrid nature of the clone (DE×D1R11), the functional properties of which are reported here, was documented by cytogenetic analysis and HLA phenotyping. The cells showed a stable hypotetraploid karyotype after 15 days to 6 months of continuous culture. Moreover, the presence of two morphologically distinct Y chromosomes, each from one parental cell, was shown using the quinacrine fluorescence technique (QFD) (Fig. 1). Although HLA typing of these hybrid cells was difficult because of their susceptibility to complement, they expressed definitely the HLA-A₂ antigen from DE, which was absent from the parental line, together with HLA antigens from KE37.

The hybrid line was studied for expression of several T-cell markers. The percentage of E rosette-forming cells was low in the D1R11 clone and varied from 25 to 75% in the hybrid line. However, when observed by immunofluorescence using a monoclonal antibody (OKT₁₁) to the E rosette-receptor, all DE×D1R11 cells were positive whereas <5% of cells of the parental cell line were stained. The staining by indirect immunofluorescence of DE×D1R11 cells using a rabbit antiserum to peripheral T cells¹¹ was much stronger than that of D1R11 cells. Additional studies were done using monoclonal antibodies to T cells (Table 2). Note that T6 (a common thymocyte antigen), which was absent on DE but present on D1R11 cells, was found on all hybrid cells. The T8 antigen, which was present on only a small fraction of D1R11 cells but found on most normal parental T cells, was expressed on all the hybrid cells. In contrast to T8, T5 antigen was absent from the hybrids although found together with T8 on DE lymphocytes (T5 and T8 define the suppressor/cytotoxic T-cell phenotype).

T3 antigen was not detected on the hybrids although it is always associated with T5 and T8 on normal PBL as well as on the DE cells which were used in the fusion. Similarly, Ia antigens were not detectable by immunofluorescence on hybrids despite their expression on most DE T cells. Whether these findings reflect merely a lack of sensitivity of the assay, a chromosomal loss or possibly a complex regulation of T-cell antigen expression in hybrid cells is unknown.

Most interestingly, the hybrid line DE×D1R11 showed functional properties. As the normal cells used for fusion were obtained from a patient with agammaglobulinaemia and with a high number of circulating lymphocytes having the phenotype of activated suppressor/cytotoxic T cells (Ia, T5, T8 antigens) we determined whether the hybrids might produce a soluble factor with suppressive activity on polyclonal immunoglobulin production. As shown in Table 2, the supernatant of the hybrid line, and not that of D1R11, suppressed the generation of cytoplasmic immunoglobulin (cIg)-positive cells from normal PBL stimulated by PWM. The suppression ranged from 50 to 75% (mean 65.5%) at day 6 or 7. When tested with mono-specific antisera to the various immunoglobulin chains, the isotypic distribution of cIg cells was not modified. The suppressive effect was observed when the supernatant was added at a final concentration of 10% and was maximum at a concentration of 20%. Note that this factor was produced without any triggering of the hybrid cells.

As the stimulation of murine T hybrids by lectins may enhance the production of soluble mediators, we also tested supernatants obtained in the presence of concanavalin A ($10 \mu\text{g ml}^{-1}$). In nine such experiments (data not shown) the suppression observed with DE×D1R11 supernatant compared with that of D1R11 supernatant ranged from 55 to 90% (mean 70%), which does not differ from the above results.

Experiments now in progress will attempt to characterize the biochemical nature of this factor as well as to determine its target cell. Until now, human suppressive factors have been shown to act either on T cells or at the B-cell level¹²⁻¹⁴. No interferon was detectable in our supernatants and the hybrid cells did not carry Fc receptors for IgG, which are believed to modulate B-cell differentiation^{15,16}. Results of preliminary experiments indicate that the factor does not prevent PWM-induced mitogenesis; it has to be added in the first 36 h of culture to suppress PWM-induced B-cell differentiation, and it has no effect on B-cell

Table 2 Effects of supernatants from T-cell line (D1R11) or hybrid clone (DE×D1R11) on PWM-induced B-cell differentiation

	Expt 1		Expt 2		Expt 3		Expt 4	
	a	b	a	b	a	b	a	b
PBL with PWM	16		13		6		9	
PBL + PWM + D1R11 supernatant	17	0	11.5	11	6	0	9.6	0
PBL + PWM + DE×D1R11 supernatant	7.5	52	4	71	1.5	75	3.3	64

Peripheral blood mononuclear cells from four normal donors were cultured for 7 days in the presence of $50 \mu\text{g ml}^{-1}$ PWM with or without added supernatants (final dilution 20%) from the T-cell line D1R11 or DE×D1R11 hybrids. These supernatants were collected after 18 h culture of 2×10^6 cells ml^{-1} in DMEM plus 10% FCS. a, Percentage of cells with cytoplasmic immunoglobulin (cIg) estimated for at least 1,000 cells on day 7 of culture, detected by immunofluorescence of fixed cells treated with fluorescein-conjugated goat IgG antibody to human immunoglobulin. The number of cells and viability were similar in each case. b, Per cent suppression of the expected number of cIg cells per culture.

differentiation induced by *Nocardia*, a relatively T-independent polyclonal activator of B lymphocytes.

We have shown here that construction of hybrids with activated human T cells of homogeneous phenotype is feasible; availability of such functional T hybrids will be valuable in the production and characterization of human T-cell factors and possibly also T-cell receptors.

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- Köhler, G. & Milstein, C. *Nature* **256**, 495 (1975).
- Olsson, L. & Kaplan, H. S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 5429 (1980).
- Croce, C. M., Linnenbach, A., Hall, W., Steplewski, Z. & Koprowski, H. *Nature* **288**, 488 (1980).
- Kontinen, S. et al. *Nature* **274**, 477 (1978).
- Taniguchi, M. & Miller, J. F. A. P. *J. exp. Med.* **148**, 373 (1978).
- Taussig, M. J., Corvalan, J. R. F., Binns, R. M., Roser, B. & Holliman, A. *Eur. J. Immun.* **9**, 768 (1979).
- Takatsu, K., Tanaka, K., Tominaga, A., Kumahara, Y. & Hamaoka, T. *J. Immun.* **125**, 2646 (1980).
- Katz, D., Bechtold, T. & Altman, A. *J. exp. Med.* **152**, 956 (1980).
- Littlefield, J. W. *Science* **145**, 709 (1964).
- Reinherz, E. L. & Schlossman, S. F. *Cell* **19**, 821 (1980).
- Brouet, J. C. & Chevalier, A. *J. Immun.* **122**, 260 (1979).
- Fleisher, T. A., Greene, W. C., Blaese, R. M. & Waldmann, T. A. *J. Immun.* (in the press).
- Wolf, R. L., Whitsed, H., Rosen, F. S. & Merler, E. *Cell. Immun.* **36**, 231 (1978).
- Saxon, A. & Stevens, R. H. *Clin. Immun. Immunopath.* **10**, 427 (1978).
- Broder, B. R. & Merigan, T. C. *J. Immun.* **113**, 1319 (1974).
- Lethibichthuy, Samarut, C., Brochier, J., Fridman, W. H. & Revillard, J. P. *Eur. J. Immun.* **10**, 894 (1980).

Switch region of immunoglobulin C_μ gene is composed of simple tandem repetitive sequences

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Immunoglobulin heavy (H) chain genes comprise a family of variable region (V) genes and several constant region (C) genes which are classified, in mouse, into five major classes: μ , γ , α , δ and ϵ . During differentiation of a given B lymphocyte, a specific V_H gene is first expressed as a part of the μ -chain and at a later stage the expressed H chain switches the C region from μ to γ or α without alteration of the V_H region sequence¹⁻³. This phenomenon, called immunoglobulin class switch, involves a unique recombination event that takes place at the region 5' to each C_H gene during B-lymphocyte differentiation⁴⁻⁶. The

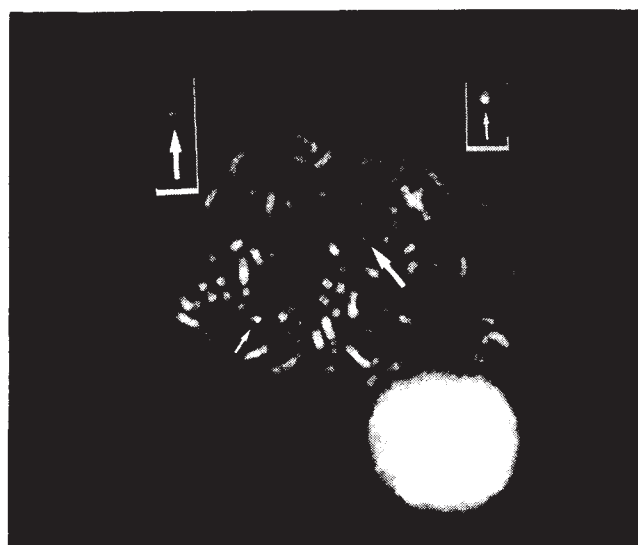


Fig. 1 Hypotetraploid (78 chromosomes) metaphase of DE×D1R11 hybrid clone. QFD staining shows two different Y chromosomes: one long chromosome from the T-cell line (main picture small arrow) and the second, small one originating from DE cells (main picture, large arrow). Inset right, DE×D1R11 long Y chromosome; inset left, DE×D1R11 small Y chromosome.

Fig. 2 Nucleotide sequence of the S_{μ} region. Nucleotide sequences are displayed from left to right with the direction of transcription of the structural sequence. The sequences are punctuated so that GGGGT is aligned at the same position in each repeating unit. DNA sequence was determined according to the method of Maxam and Gilbert¹⁵ with slight modifications²⁶. DNA fragments to be sequenced were digested with the enzymes indicated in Fig. 1, treated with bacterial alkaline phosphatase (Boehringer) and phosphorylated with T4 polynucleotide kinase (New England Biolabs) and [γ -³²P]ATP (Amersham). ³²P-labelled DNA fragments were isolated by polyacrylamide gel electrophoresis and cleaved with second restriction enzymes. Fragments labelled only at one end were isolated by polyacrylamide gel electrophoresis and purified as described previously²⁶. The regions indicated I, II and III are shown in Fig. 1. Region II was determined using rearranged $\gamma 3$ gene of a $\gamma 3$ -chain-producing myeloma J606 (unpublished data). Arrow *a* indicates the recombination site of IF-2¹⁰ while arrows *b* and *c* indicate the deletion site of MEP203¹².



of rather homogeneous repetitive sequences, with GAGCT and GGGGT as basic units.

Dunnick *et al.*¹⁰ found repetition of (GAGCT)₃(GGGGT) in the 5'-flanking region of the variant $\gamma 1$ gene of a $\gamma 1$ -chain-producing myeloma, IF2. As this $\gamma 1$ gene has lost a large segment (5.5 kb) of DNA, including the C_H1 domain, a part of the 5'-flanking region of the C_γ1 gene and portions of the S_μ and S_γ1 regions, these workers were unable to locate the germ-line origin of (GAGCT)₃(GGGGT) sequence. Nonetheless, they have discussed the possible role of these sequences in the class switch. Comparison of their sequences with ours indicates that the 5' deletion point of the IF2 $\gamma 1$ gene is located in the S_μ region at arrow *a* shown in Fig. 2 (also see Fig. 1).

In addition to three class switch recombination sites determined previously^{6,8,9,12,13}, we have recently determined two more such sites in the $\gamma 3$ -gene clone isolated from J606 myeloma and the ϵ -gene clone (IgE1) isolated from IgE-producing hybridoma²⁸ (see Fig. 1). The class switch sites of various classes are ordered as 5'-M141($\gamma 2b$)-T15(α)-Ige-1(ϵ)-MC101($\gamma 1$)-J606($\gamma 3$)-3'. Although these recombination sites tend to cluster at the 5' side of the S_μ region, one cannot deduce any physiological significance from this fact because it is possible that a secondary deletion of the S region surrounding the recombination sites occurs during the propagation of myelomas and hybridomas. In fact, the α gene of MOPC603 has an internal deletion within the S_μ region⁸. The 5' recombination site in the S_μ region was located (see Fig. 1) but the 3'-recombination site of the MOPC603 gene, which is the actual class switch recombination site, is not known. Comparison of the nucleotide sequences surrounding each class switch recombination site seems to indicate that H-chain switching is not highly specific to the nucleotides joined⁷ (T.N., in preparation). Again, this interpretation is limited if there has been secondary deletion around recombination sites.

We and others have observed that a major portion of the 3.7-kb *Hind A* fragment is deleted during its propagation in *Escherichia coli*^{4,5,11,12}. Such deletion seems to depend on the

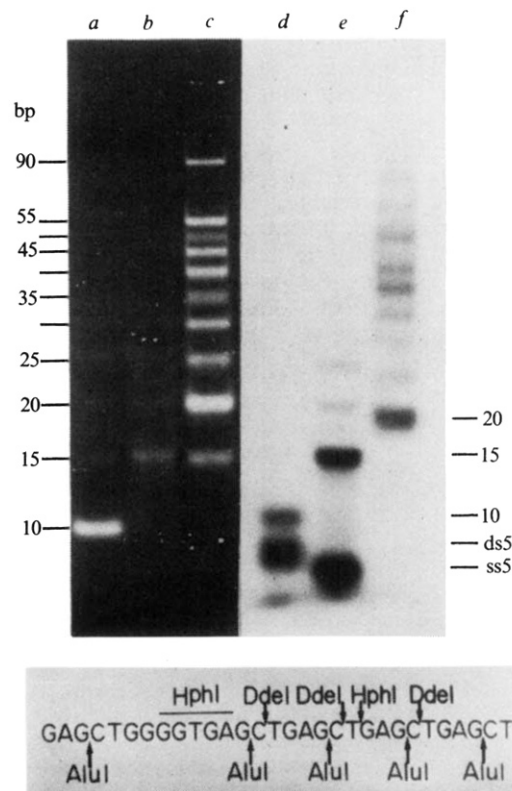


Fig. 3 Digestion of the S_μ region with restriction enzymes. 0.6 μ g of 2.6-kb *Sau3A* fragment was cleaved with *AluI* (*a*), *DdeI* (*b*) or *HphI* (*c*), electrophoresed in 10% polyacrylamide gel and stained with ethidium bromide. The sizes of fragments are shown in base pairs. 0.6 μ g of the 2.6-kb *Sau3A* fragment was labelled with ³²P-dCTP by nick translation²⁹ and cleaved with *AluI* (*d*), *DdeI* (*e*) or *HphI* (*f*). The sizes of the fragments were determined with pBR322 DNA digested with *ThaI* or *AluI* as standard. The 5-bp fragment of *DdeI* digests ran faster than that of *AluI* digests, probably because the former has only 2 bp and denatures easily. Nucleotide sequences of restriction sites of the enzymes used are shown at the bottom. ds, Double stranded; ss, single stranded.

host strain; for example, deletion was very frequent in LE392 but rare in C600 regardless of whether they were *recA*⁻ or *recA*⁺. The deletion seems to take place at limited sites (indicated by A, B, C and D in Fig. 1). The cloning of the *Hind* A fragment in pBR322 with a host strain of LE392 has yielded 12 variant clones with inserts of different sizes. The deleted fragment was one of 2.8 kb between sites A and D, 2.3 kb between A and C, 2.4 kb between B and D or 1.9 kb between B and C. Embryonic *C_μ*-gene clone MEP203¹² deleted the 2.8-kb DNA fragment between arrows *b* and *c* in Fig. 2. The nucleotide sequences surrounding the deletion sites of A, B, C and D seem to correspond to the regions containing tandem repetition of regular 20-bp units (Fig. 2).

The S region was originally defined as the functional region responsible for the class switch recombination. We have shown that the structural basis of the S_γ region is tandem repetition of homologous 49-bp units⁷. The S_α region contains tandem repetition of 80-bp units^{8,9}. It is now clear that tandem repetitive sequences of 20–30-bp units are present in the 5'-flanking region of the *C_μ* gene where the class switch recombination occurs. These nucleotide sequences contain abundant short sequences such as GAGCTG and TGGGG which are shared by repeating sequences in the S_{γ3}, S_{γ1}, S_{γ2b} and S_α regions as shown in Table 1. It is known that the tandem repetitive sequence increases the chance of homologous recombination¹⁶. Accordingly, the recombination responsible for class switch from *μ* to *α* or from one *γ* to another *γ*, may be facilitated by such homology of repeating sequences occurring widely in the S region. Tens of short common sequences may be sufficient to make a transient join between two DNAs when they are brought close to each other. As the nucleotide sequences of the repeat units of the S regions are different, it is possible that specific enzymes catalyse the S–S recombination for different pairs of S regions. Both V–J (joining) and S–S recombinations are accompanied by the deletion of intervening DNA segments^{17–24}. We have recently proposed a model in which S–S recombination is mediated by an unequal crossing-over event between sister chromatids⁹.

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- Nossal, G. J. V., Warner, N. L. & Lewis, H. *Cell. Immun.* **2**, 41–53 (1971).
- Pernis, B., Fornì, L. & Amante, L. *Ann. N.Y. Acad. Sci.* **190**, 420–431 (1971).
- Cooper, M. D., Kearney, J. F., Lydyard, P. M., Grossi, C. E. & Lantoni, A. R. *Cold Spring Harb. Symp. quant. Biol.* **41**, 139 (1976).
- Kataoka, T., Kawakami, T., Takahashi, N. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **77**, 919–923 (1980).
- Davis, M. M. *et al. Nature* **283**, 733–739 (1980).
- Maki, R., Traunecker, A., Sakano, H., Roeder, W. & Tonegawa, S. *Proc. natn. Acad. Sci. U.S.A.* **77**, 2138–2142 (1980).
- Kataoka, T., Miyata, T. & Honjo, T. *Cell* **23**, 357–368 (1981).
- Davis, M. M., Kim, S. K. & Hood, L. *Science* **209**, 1360–1365 (1980).
- Obata, M. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 2437–2441 (1981).
- Dunnick, W., Rabbitts, T. H. & Milstein, C. *Nature* **286**, 669–675 (1980).
- Marcu, K. B., Banerji, J., Pennicavage, N. A., Lang, R. & Arnheim, M. *Cell* **22**, 187–196 (1980).
- Sakano, H., Maki, R., Kurosawa, Y., Roeder, W. & Tonegawa, S. *Nature* **286**, 676–683 (1980).
- Takahashi, N., Kataoka, T. & Honjo, T. *Gene* **11**, 117–127 (1980).
- Kawakami, T., Takahashi, N. & Honjo, T. *Nucleic Acids Res.* **8**, 3933–3945 (1980).
- Maxam, A. M. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 560–564 (1977).
- Farabaugh, P. J. & Miller, J. H. *J. molec. Biol.* **126**, 847–863 (1978).
- Honjo, T. & Kataoka, T. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2140–2144 (1978).
- Yaoita, Y. & Honjo, T. *Nature* **286**, 850–853 (1980).
- Yaoita, Y. & Honjo, T. *Biomed. Res.* **1**, 164–175 (1980).
- Coleclough, C., Cooper, C. & Perry, R. P. *Proc. natn. Acad. Sci. U.S.A.* **77**, 1422–1426 (1980).
- Cory, S., Jackson, J. & Adams, J. M. *Nature* **285**, 450–456 (1980).
- Rabbitts, T. H., Forster, A., Dunnick, W. & Bentley, D. L. *Nature* **283**, 351–356 (1980).
- Sakano, H., Huppi, K., Heinrich, G. & Tonegawa, S. *Nature* **280**, 288–294 (1978).
- Seidman, J. G. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 6022–6026 (1980).
- Clewell, D. & Helinski, D. *Proc. natn. Acad. Sci. U.S.A.* **62**, 1159–1166 (1969).
- Honjo, T. *et al. Cell* **18**, 559–568 (1979).
- Kataoka, T., Yamawaki-Kataoka, Y., Yamagishi, H. & Honjo, T. *Proc. natn. Acad. Sci. U.S.A.* **76**, 4240–4244 (1979).
- Nishida, Y. *et al. Proc. natn. Acad. Sci. U.S.A.* **78**, 1581–1585 (1981).
- Maniatis, T., Jeffrey, A. & Kleid, D. K. *Proc. natn. Acad. Sci. U.S.A.* **72**, 1184–1188 (1975).

Natural killer cells kill tumour cells at a given stage of differentiation

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Natural killer (NK) cells have unique surface features and physiological characteristics and a selective ability to lyse some, but not other, target cells^{1,2}. However, the basis of this selectivity remains obscure at both effector and target cell levels. Proposed specific NK-cell target moieties^{3–5} include glycolipids, and glycoproteins unrelated to the major histocompatibility complex, while malignant and certain normal cells have been found to be susceptible to NK-cell-mediated cytotoxicity^{6–8}. There is good evidence that NK cells can inhibit the outgrowth of small numbers of transplanted tumour cells *in vivo*^{9,10} and can restrict the establishment of secondary metastasis^{11,12}. It has thus been speculated that NK cells function as a primitive, thymus-independent immune system using phylogenetically preserved target structures to form a cell-mediated resistance barrier against the outgrowth of certain tumour cells¹. The presence in the thymuses of neonatal mice and humans^{8,13} and in the marrow of human fetuses, of apparently normal cells which are quite sensitive to NK cells suggested that NK cells might have an increased ability to cause the lysis of cells at a particular stage of their differentiation. In agreement with this concept, embryonal carcinoma cells at various stages of differentiation display a strikingly different susceptibility to NK-cell-induced lysis¹⁴. Here only cell types representing early stages were sensitive to NK-cell-mediated cytotoxicity, whereas the more differentiated endodermal cell lines showed close to complete resistance. The above data would thus support the view that, *in vivo*, depending on the stage of differentiation, both normal and malignant cells are under surveillance by NK cells. Here we have considered the question of differentiation-related NK-cell susceptibility using defined cell lines known to undergo controlled differentiation in the presence of various agents. Three tumour cell-lines were investigated, and all demonstrated a striking positive correlation between a decrease in NK-cell susceptibility and the induction of differentiation by the various inducers. Our findings support the view that susceptibility to NK-cell mediated lysis may vary according to the stage of differentiation of the target cell.

Table 1 shows the three cell lines used in the present study and the impact of the inducing agents on NK-cell sensitivity in relation to various differentiation markers. The NK-cell sensitivity of K-562, the target cell line most commonly used to assess human NK-cell activity, decreased after exposure to sodium butyrate or haemin. This loss was well correlated in time and dose of the respective inducer with an increase in erythroid differentiation markers such as the expression of surface glycoprotein A and haemoglobin synthesis¹⁵. U-937 is a NK-cell-sensitive human histiocytic lymphoma cell line¹⁶. The phenotype of this cell line suggests that it represents monocytic cells arrested at a differentiation stage close to the myelomonocytic stem cell^{17,18}. Treatment of U-937 cells with either 12-*O*-tetradecanoyl phorbol acetate (TPA) or supernatant from 5-day mixed lymphocyte cultures (MLC) resulted within 2–3 days in the appearance of 30–60% of cells with a macrophage-like phenotype^{18–20}. Concomitantly, a drastically reduced NK-sensitivity profile was noted. K-562 and U-937 show a similar loss of sensitivity regardless of the type of inducer used, demonstrating that 'unrelated' agents giving the same cellular

Table 1 Effect of differentiation-inducing agents on NK-cell-susceptible target cells

Cell line	Inducer	Non-induced		Induced		Phenotypic alterations during induced differentiation ^{18, 21}
		Effector: target cell ratio				
		50:1	6:1	50:1	6:1	
K-562 (human)	BA	49.6	25.2	24.8	8.6	Benzidine staining reaction becomes positive Plasma membrane: specific changes in glycoprotein composition, increase in glycophorin A
K-562 (human)	Haemin	52.6	22.8	29.6	6.0	Function: globin production
U-937 (human)	MLC supernatant	38.0	26.5	16.6	8.5	Morphology: acquisition of a macrophage-like morphology Plasma membrane: specific changes in surface glycoprotein composition, increase in HLA and Ia-like antigen; β_2 -microglobulin and Fc receptors
U-937 (human)	TPA	21.4	7.3	2.9	0.0	Cytoplasmic enzymes: increase in nonspecific esterases Function: increased capacity for phagocytosis and lysozyme secretion, induced activity as effector cell in ADCC
GM-86 (mouse)	HBMA	36.7	20.4	18.1	2.4	Function: increase in globin production

K-562, a human erythroleukaemia cell line¹³, was cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and antibiotics. U-937, a 'true' histiocytic human cell line¹⁷, was cultured in F10 supplemented as above. GM-86 (clone 745), a murine Friend virus-induced leukaemia originating from DBA/2J (ref. 21), was grown in Eagle's medium supplemented with 20% FCS and antibiotics. Cells were tested for NK-cell sensitivity using Ficoll-Isopaque-isolated human peripheral blood cells when testing K-562 and U-937, and mouse (CBA/H) spleen cells when analysing GM-86. The assay used was a 4-h ⁵¹Cr-release test performed in microtitre plates with a fixed number of target cells (10^4 cells) and various effector cell concentrations as previously described¹⁰, using RPMI 1640 supplemented with 10% newborn calf serum and 10 mM HEPES. Calculations were done accordingly to the formula (c.p.m. in test - c.p.m. in spontaneous control)/(c.p.m. in detergent - c.p.m. in spontaneous control). Before assay, target cells were treated as follows. Each tumour cell population was split in half at day 0, one half being cultured in the presence of inducer, the other half serving as control in appropriate media. K-562 cells were treated with sodium butyrate at 1 mM for 3 days to give optimal differentiation²⁰. Haemin was added at 10^{-4} M and cells were cultured for 5 days. U-937 cells were cultured in medium supplemented with 20% day-6 supernatant prepared as described elsewhere^{19, 20}. Control cells were cultured in 20% supernatant derived from responder cells alone. Cells were seeded for optimal growth and differentiation at an initial concentration of 3×10^5 cells ml^{-1} and further cultured for 3 days²⁰. For TPA treatment cells were seeded at 3×10^5 cells ml^{-1} and grown in the presence of 1.6×10^{-7} M of TPA for 3 days as described elsewhere¹⁸. Mouse GM-86 cells were grown in the presence of 4 mM HMBA for 5 days²¹. After induction the cells were washed free of inducer and labelled with ⁵¹Cr as described elsewhere¹⁰. Cell viability throughout was judged by Trypan blue dye exclusion and was never <80%. No growth inhibition was noted for K-562 or GM-86. U-937 showed ~50% reduction in cell number. Each titre point in the cytotoxic assay was done at least in triplicate, and standard error did not exceed 5% of the mean. Data represent one of at least three similar experiments.

alterations also induce a similar increase in NK-cell resistance. Finally, following treatment with hexametabisacetamide (HMBA), GM-86, a mouse Friend virus-transformed erythroid stem cell line²¹, acquired the capacity for globin production. As with the differentiated K-562 and U-937 cell lines, a simultaneous decrease in NK-cell sensitivity was noted.

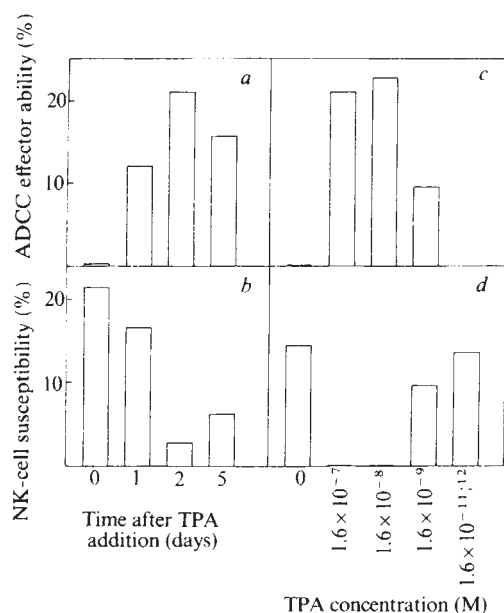


Fig. 1 Time and dose correlation between induced differentiation and loss of NK-cell susceptibility of U-937 cells. *a, b*, Time correlation. U-937 cells were cultured with TPA as in Table 1 for the indicated period of time. Induction was initiated at different times to allow simultaneous collection at day 5. After cultivation the cell populations were washed and split. One part was tested for NK-cell susceptibility as in Table 1. The other part was used as effector cells in an ADCC test against antibody-coated chicken red blood cells (CRBC) in a 4-h ⁵¹Cr-release test in microtitre plates as described elsewhere². A rabbit anti-CRBC IgG fraction was used at 1.2×10^{-4} final dilution. Effector:target ratio for assaying tumour susceptibility was 50:1 and for ADCC-ability determination 5:1. Data represent one of two similar experiments. *c, d*, Dose correlation. U-937 cells were cultured in the indicated concentrations of TPA for 3 days and tested as above. Data represent one of two similar experiments.

Figure 1 shows the strong parallelism seen between the time and dose effect of TPA on U-937 cells with respect to induced differentiation (for other differentiation markers see Table 1), as exemplified by development of the ability to exert antibody-dependent cellular cytotoxicity (ADCC) and by the loss of NK-cell sensitivity. The highest degree of cytotoxic effector potential was found on days 2–3 (Fig. 1*a*), at which time the tumour cell populations also showed the lowest degree of NK-cell sensitivity (Fig. 1*b*). Doses of TPA inducing maximal ADCC ability, scored on day 3 (Fig. 1*c*), similarly produced a highly NK-cell-insensitive cell population (Fig. 1*d*).

Exposure of BM-86 cells for different periods of time to HMBA, in concentrations inducing optimal erythroid differentiation, resulted in a loss of NK-cell sensitivity (Fig. 2*a*) which was accompanied by the appearance of globin synthesis as detected by SDS polyacrylamide gel analyses of lysates of GM-86 cells labelled with ³⁵S-methionine in identical conditions (Fig. 2*c*). In Fig. 2*b* the same GM-86 cell populations as in Fig. 2*a* were used as cold target competitors against the well known mouse NK target cell YAC-1 (ref. 6). The time-dependent decrease in NK-cell sensitivity (Fig. 2*a*) was paralleled by a gradual loss of competing ability. We interpret this to mean that the decreased NK-cell susceptibility of the GM-86 cells after induced differentiation is due to a loss of binding structure(s) for NK cells. However, a similar decrease in competing ability was not seen using differentiated U-937 cells in a parallel system (data not shown). This implies that the differentiation-related alterations seen on the target cell can be expressed either at the level of binding or as a change in susceptibility to lysis. The present system may thus allow the binding and lytic events to be distinguished at the NK target cell level.

The kinetics of the events argue against the possibility that the inducing agents *per se* cause a decrease in NK-cell sensitivity by some alteration of the target cell, not related to the induced differentiation. This possibility was further reduced by results obtained using spontaneous subclones of K-562 which, as judged by their higher expression of cell-surface glycophorin A, represented a more advanced stage of erythroid differentiation than the original K-562. As Fig. 3*a* shows, such subclones were also significantly more resistant to NK cytotoxicity. The clones and original populations could be further differentiated with sodium butyrate to reach the same, still lower, degree of NK-cell sensitivity, indicating the efficiency of the agent in promoting

differentiation to the same final stage in the three different K-562 populations.

To investigate further the correlation between the expression of a differentiation marker and the NK-cell sensitivity profile of cells within a non-cloned tumour cell line, TPA-treated U-937 cells were labelled with fluorescein isothiocyanate (FITC) rabbit anti-human HLA-DR IgG (Fab')₂ fragments and sorted on a FACS II cell sorting machine. The population of cells showing markers indicative of greater differentiation (for example, large size and increased expression of HLA-DR¹⁸) were completely resistant to NK-cell lysis (Fig. 3b).

In conclusion, the human K-562 and U-937 cell lines and the mouse GM-86 tumour show a clear correlation between the expression of markers for induced differentiation and reduced NK-cell sensitivity. However, we do not know whether NK-cell sensitivity is always restricted to target cells at an early stage in their respective differentiation. In fact, examples of highly differentiated tumour cells such as myeloma cells, which are quite susceptible to NK lysis¹⁶, the wide variability of NK-cell sensitivity among tumours of the same histogenetic type¹⁰ and the fact that treatment of tumour cells with sodium butyrate can in some cases lead to increased NK-cell sensitivity²⁴, strongly argue against too simplistic a view in this regard. The results do, however, demonstrate that the varying differentiation stages expressed by a particular cell type in a defined differentiation pathway are important in determining NK-cell susceptibility. The availability of the present *in vitro* systems in which controlled differentiation can be induced significantly increases

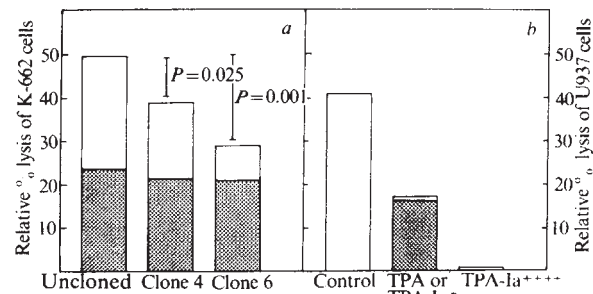


Fig. 3 The reduction in susceptibility of K-562 and U-937 cells is associated with more mature cells. *a*, Analysis of agarose-cloned K-562 cells. K-562 cells were cloned in agarose and cloned sublines analysed for the expression of surface glycoporphin A by immunofluorescence using a rabbit anti-human glycoporphin A antiserum (provided by Dr L. Andersson). Sublines expressing the highest amounts of glycoporphin A as determined by quantitative cytofluorometric methods²³ were further subcultured. The original K-562 population and its cloned derivatives were tested as NK-cell targets before and after treatment with sodium butyrate (butyrate treatment hatched area) at a 50:1 ratio as in Table 1. Simultaneously the surface expression of glycoporphin A was quantitated as described above. The *P* value after butyrate treatment was >0.5. Data represent one of two similar experiments within a 3-week interval. The *P* value was calculated as in Fig. 2. *b*, U-937 cells were induced with TPA as described in Table 1 legend. The cells were then labelled with ⁵¹Cr and incubated with an FITC-labelled rabbit F(ab')₂ anti-HLA-DR antiserum (provided by Dr L. Klarskog) using phosphate-buffered saline with 0.02% NaN₃ for 30 min on ice at a dilution of 1:5. Cells were then washed once in the same buffer and sorted at 4 °C on a FACS II sorter (Becton and Dickinson). Windows were set to obtain the upper 15% of the large, strongly fluorescent cells, denoted TPA-la⁺⁺⁺, giving 6.5 × 10⁴ cells in the right channel. Small cells with insignificant fluorescence were obtained in the left window channel (left channel = 39 × 10⁴ cells), cutting out debris and dead cells (<10% of total). Cells were washed again after sorting, counted, diluted and used as targets in a short-term NK-cell lysis as described in Table 1 legend using a 50:1 effector:target ratio. Control = cells left in culture without TPA and not sorted; TPA = cells induced to differentiate with TPA but not sorted; TPA-la⁺⁺⁺ = cells induced to differentiate with TPA followed by FACS-II sorting using the right channel. Hatched area = sensitivity of TPA-treated, weak la⁺ cells (left channel, 79% of total TPA cells are TPA-la⁺).

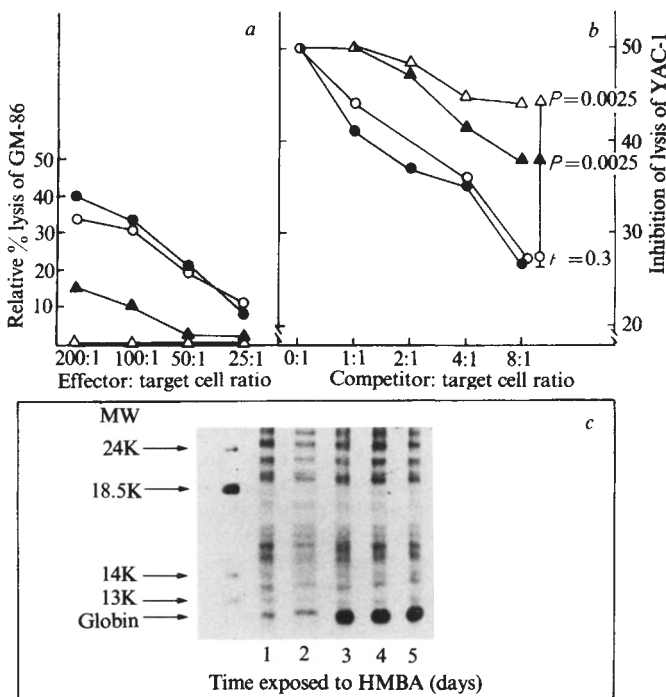


Fig. 2 Induced differentiation in mouse GM-86 cells is accompanied by a loss in NK-cell susceptibility and NK-target competing ability. *a*, GM-86 cells were cultured with HMBA as in Table 1 for 0 (●), 2 (○), 3 (▲) and 5 (△) days. The cells were then collected and washed; one part was used as targets for mouse spleen cells as in Table 1, the other part was used (*b*) as cold competitors against the mouse NK target, the T lymphoma YAC-1, where indicated amounts of cold, unlabelled GM-86 target cells were added to a fixed 1:100 ratio of ⁵¹Cr-labelled YAC-1 target to mouse spleen cells to test for lysis as in Table 1. *P* values were calculated using paired *t*-statistics making versus day 0 values. *c*, GM-86 cells grown in identical conditions as in *a* and *b* were pulse labelled with ³⁵S-methionine²². The lysates representing each time point were analysed by polyacrylamide gel electrophoresis as described elsewhere²². The globin band was confirmed by immunoprecipitation with a specific rabbit anti-mouse globin antibody (M.J., unpublished). MW, molecular weight.

the possibility of analysing fine target structures relevant to NK-cell-mediated cytotoxicity at the level of binding and/or during the actual lytic event.

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- Kiessling, R. & Wigzell, H. *Immun. Rev.* **44**, 165–208 (1979).
- Herberman, R. B. (ed.) *Natural Cell-Mediated Immunity Against Tumours* (Academic, New York, 1980).
- Roder, J. C., Åhrlund-Richter, L. & Jondal, M. *J. exp. Med.* **150**, 471–480 (1979).
- Young, W. W., Durdik, J. M., Urdal, D., Hakomori, S.-I. & Henney, C. S. *J. Immun.* **126**, (1981).
- Kiessling, R. & Wigzell, H. *Curr. Topics Microbiol. Immun.* (in the press).
- Kiessling, R., Klein, E. & Wigzell, H. *Eur. J. Immun.* **5**, 112–117 (1975).
- Cudkovic, G. & Hochman, P. S. *Immun. Rev.* **44**, 13–41 (1979).
- Hansson, M., Kiessling, R., Andersson, B., Kärre, & Roder, J. *Nature* **278**, 174–176 (1979).
- Haller, O., Hansson, M., Kiessling, R. & Wigzell, H. *Nature* **270**, 609–611 (1977).
- Riesenfeldt, I. *et al. Int. J. Cancer* **25**, 399–403 (1980).
- Talmadge, J., Meyers, K., Prieur, D. & Starkey, J. *Nature* **284**, 622–624 (1980).
- Hanna, N. & Fiddler, I. *J. natn. Cancer Inst.* (in the press).
- Hansson, M. & Kiessling, R. in *NK Cells: Fundamental Aspects and Role in Cancer* (ed. Herberman, R. B.) (North-Holland, Amsterdam, in the press).
- Stern, P., Gidlund, M., Örn, A. & Wigzell, H. *Nature* **285**, 341–342 (1980).
- Andersson, L. C., Jokinen, M. & Gahmberg, C. G. *Nature* **278**, 364–365 (1979).
- Pattengale, P. K. *et al. Int. J. Cancer* (in the press).
- Sundström, C. & Nilsson, K. *Int. J. Cancer* **17**, 565–577 (1976).
- Nilsson, K. *et al. Mod. Trends Hum. Leukaemia* **26** (in the press).
- Koren, H. S., Andersson, S. J. & Larick, J. W. *Nature* **279**, 328–331 (1979).
- Nilsson, K., Andersson, L. C., Gahmberg, C. G. & Forsbeck, K. in *New Trends in Human Immunology and Cancer Immunotherapy* (eds Serrou, B. & Rosenfeld, C.) 271–282 (Doin, Paris, 1981).
- Gazit, Y., Reuben, R., Deitch, A., Marks, P. & Ritkind, R. *Cancer Res.* **38**, 3779–3785 (1978).
- Persson, H., Hansson, M. & Philipson, L. *J. molec. Biol.* **136**, 375–394 (1980).
- Nilsson, K., Evrin, P. E. & Welsh, K. I. *Transplant. Rev.* **21**, 53–84 (1974).
- Blazar, B., Patatroyo, M., Klein, E. & Klein, G. *J. exp. Med.* **151**, 614–627 (1980).

Cloned copy of the haemagglutinin gene codes for human influenza antigenic determinants in *E. coli*

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The haemagglutinin (HA) of influenza virus is the major antigen towards which neutralizing antibodies are directed¹. Epidemics and pandemics of influenza are associated with variation in the structure of HA^{2,3}. We have recently cloned and sequenced a DNA copy of the RNA gene coding for the HA from human strain A/Japan/305/57 (H2 subtype)^{4,5}. Recent advances in recombinant DNA technology have made it feasible to express in bacteria proteins coded by eukaryotic genes. Viral antigens synthesized in bacteria may ultimately be useful as vaccines. We now report the expression in *Escherichia coli* of HA antigenic determinants which might be the first approach towards production of a human vaccine. Expression was obtained from the cloned HA gene inserted into a plasmid under the control of the *E. coli lac* promoter.

Plasmid pJHA⁵ contains a copy of the entire Japan HA gene except for the terminal 11 nucleotides of the 5' non-translated region of the viral RNA. The gene contains an uninterrupted coding sequence specifying a protein of 562 amino acids including the amino-terminal hydrophobic signal peptide of 15 amino acids.

The plasmid pOP203-13 (ref. 6) contains a 203-base pair (bp) DNA sequence consisting of the *E. coli lac* UV5 promoter, ribosome-binding site and codons for the first seven amino acids of β -galactosidase. This *lac* promoter will direct transcription of any DNA fragment inserted into the single *EcoRI* site of the plasmid (see Fig. 1).

The convenient location of a single *BamHI* restriction site immediately after the AUG start codon in the HA gene⁵ allows the construction of a chimaeric plasmid which should code for a fusion protein consisting of the N-terminal seven amino acids of β -galactosidase followed by the complete HA amino acid sequence. The steps in this construction, together with the N-terminal sequence of the expected fusion protein, are shown in Fig. 1. *E. coli* HB 101 was transformed with the chimaeric plasmids, and ampicillin-resistant clones containing HA gene sequences were detected by hybridization with ³²P-labelled HA DNA⁷. The orientation of the inserts was determined by restriction enzyme digestions of plasmid DNA isolated from 24 positive clones. In 13 clones the inserts were in the correct orientation for expression with respect to the *lac* operator-promotor. Expression of HA antigenic determinants was analysed by solid-phase radioimmunoassay (RIA) using rabbit anti-Japan HA immunoglobulin. As shown in Fig. 2, plasmids pOR4, pOR9 and pOR19 expressed significant amounts of HA antigen.

The nucleotide sequences across the *lac*-HA junction were determined⁸ for five clones, which are shown in Fig. 3. As was expected, in the three clones (pOR4, pOR9 and pOR19) which were positive in the RIA, the HA DNA was in the correct reading frame for expression. However, they lacked HA gene sequences corresponding to the N-terminal signal peptide and amino acids up to positions 10–15 of the mature protein. Those clones in which the HA DNA was not in the correct reading frame were negative in the RIA. Analysis by restriction enzyme digestion of a further 41 clones which contained the HA insert in

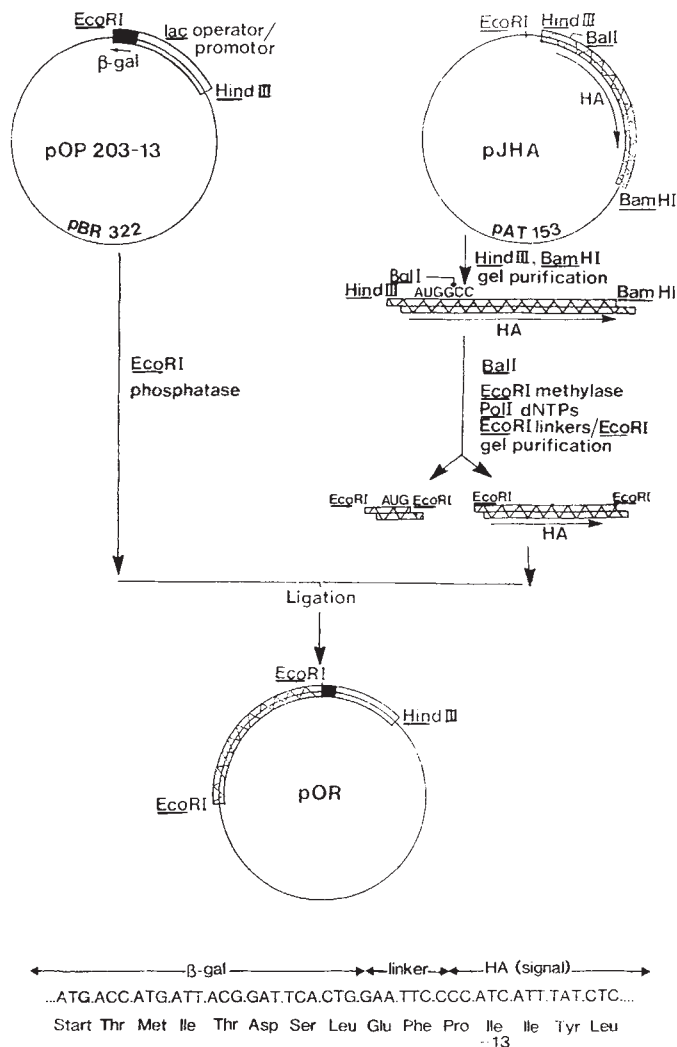


Fig. 1 Construction of expression plasmids. The *HindIII*-*BamHI* DNA fragment of pJHA⁵ (0.5 μ g) was digested with *BamHI* (restriction enzymes from New England Biolabs, used in the conditions recommended). The *EcoRI* sites of the DNA were methylated with 0.2 U of *E. coli* R1 methylase (New England Biolabs) for 1 h at 37°C. After phenol extraction and ethanol precipitation, the protruding ends of the *BamHI* and *HindIII* sites were filled using 2 U of *E. coli* DNA polymerase I (Klenow fragment; Boehringer) in a 40- μ l reaction volume containing 0.2 mM dNTPs, 60 mM Tris-HCl pH 7.5, 8 mM MgCl₂, 1.0 mM 2-mercaptoethanol, 1 mM ATP for 30 min at 10°C. 250 ng ³²P-*EcoRI* linkers (Collaborative Research; labelled with ³²P-ATP using polynucleotide kinase) in 15 μ l of the above buffer were added and ligated to the DNA using 0.3 U of T4 DNA ligase and 1 U of T4 RNA ligase for 16 h at 9°C. The buffer was then adjusted to 100 mM Tris-HCl pH 7.5, 50 mM NaCl and 5 mM MgCl₂, and the DNA completely digested with *EcoRI*. The DNA fragment coding for HA was purified on a 6% acrylamide gel⁸. The plasmid pOP203-13 (200 ng) was digested with *EcoRI* and the 5'-terminal phosphates removed by treatment⁸ with 0.2 U of bacterial alkaline phosphatase in 10 mM Tris-HCl pH 9.5 for 1 h at 37°C. After phenol and chloroform extractions the DNA was ethanol precipitated and ligated to 50 ng of the HA DNA fragment for 16 h at 10°C. 0.3 ml CaCl₂-treated *E. coli* HB101 were transfected with the ligated plasmids dissolved in 0.2 ml TCM (10 mM CaCl₂, 10 mM MgCl₂, 10 mM Tris-HCl pH 7.0)¹⁷. Transformants were selected on agar plates containing 30 μ g ampicillin per ml. These colonies were transferred to nitrocellulose filters and analysed by *in situ* hybridization⁷ using ³²P-labelled HA DNA. Positive clones were picked and grown overnight in 10 ml LB broth¹⁸ containing 20 μ g ampicillin ml⁻¹ at 37°C. Mini-plasmid DNA preparations (1.5-ml cultures) were made up by a modification (Ish-Horowitz, personal communication) of the procedure of Birnboim and Doly¹⁹. Aliquots (~0.1 μ g plasmid) were taken for restriction enzyme digestions to determine the orientation of the inserts. In the HA DNA insert of pJHA there are only two *PstI* sites near the 5' end⁵, so a *PstI* digest of the pOR plasmids with inserts in the correct orientation for expression of HA will give 5,800-, 1,000- and 100-bp fragments whereas those in the wrong orientation will give 4,400-, 2,400- and 100-bp fragments. The resultant fragments were sized on a 1.7% agarose gel (data not shown).

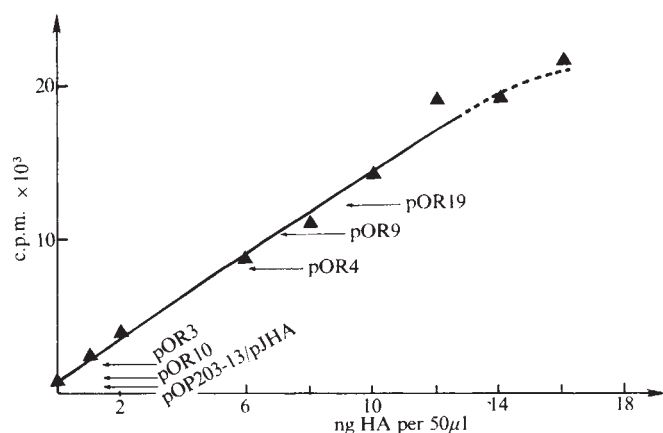


Fig. 2 Radioimmunoassay of HA in lysates of bacteria containing plasmids constructed as described in Fig. 1 legend. Bacterial lysates were prepared and assayed as described elsewhere^{10,20}. Rabbit anti-HA (IgG) was purified by chromatography on Protein A-Sepharose²¹ and labelled with ¹²⁵I using iodogen as described elsewhere²². To quantify antigen, a standard curve was constructed using different amounts of purified HA. The results were normalized to the same optical density of cells ($A_{590} = 0.5$).

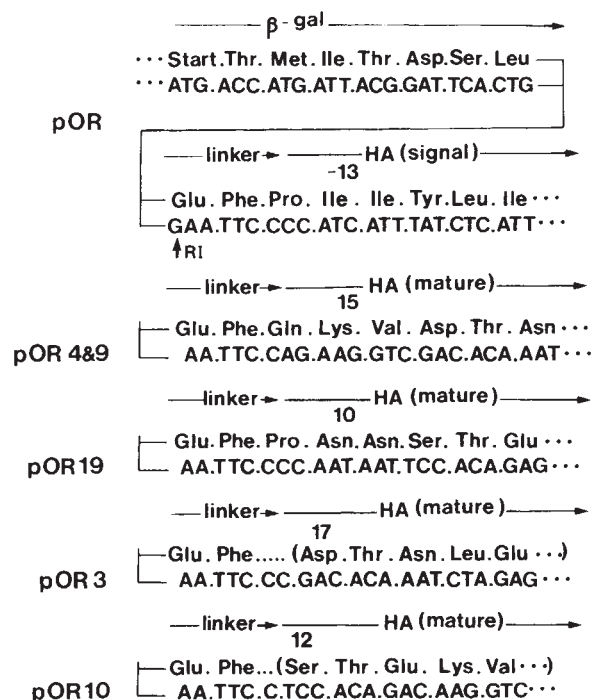


Fig. 3 Nucleotide sequences at the lac-HA junction of plasmids constructed as described in Fig. 1 legend. Only the sequence of the coding strand is given. pOR, expected sequence from the designed construction of plasmid. The nucleotide sequences were determined by the procedures of Maxam and Gilbert⁸. The plasmids were digested with *EcoRI*, treated with calf intestinal phosphatase (Boehringer) to remove the terminal 5'-phosphates²³ and then radiolabelled with ³²P-ATP using T4 polynucleotide kinase (PL Biochemicals). The fragments from a subsequent restriction digest with *AvaI* were separated on a 6% acrylamide gel and those containing the HA insertion point were eluted and sequenced.

the correct orientation did not yield any which contained sequences corresponding to the signal peptide or the entire mature HA protein, and showed that in no case had the clones lost any nucleotide sequences corresponding to the C-terminus of the protein. When the construction of the chimaeric plasmid was repeated with the modification that the *EcoRI* methylation step preceded the *BalI* digestion, the only clones with the HA insert in the correct orientation with respect to the *lac* promoter had small insertions at the *lac*-HA junction (results not shown). The reason for the difficulty in obtaining the desired construction is not yet clear. The possibility that the eukaryotic hydrophobic signal sequence of the HA protein is in some way inhibitory or toxic for growth of *E. coli* is being investigated.

The level of expression in pOR19 corresponds to ~3,000 molecules of HA per cell. Quantification by RIA assumes that the antigenicity of the hybrid protein synthesized in *E. coli* is identical with that of the natural HA. This may not be the case as the hybrid protein lacks some amino acids normally at the N-terminus of the mature HA even though they are remote from the major antigenic sites on the molecule⁹. In addition, the three-dimensional structure of the hybrid protein may differ from that of the natural protein if the correct folding requires transport through a membrane and glycosylation. These factors may affect both the antigenicity and the stability of the bacterially produced protein so that the above estimate of expression level will be a minimum value.

This is the first report of expression of a human influenza HA in bacterial cells. Antigenic determinants of the HA of a related avian orthomyxovirus, fowl plague virus, have been expressed in bacteria using a tryptophan operator/promotor system¹⁰. The level of expression of HA from our plasmid pOR19 is comparable with that of pWT111/FPV502 (ref. 10), although higher levels of expression were obtained after derepression of the *trp* promoter. The expression level is also comparable with those reported for other eukaryotic genes expressed in *E. coli*^{6,11-16}. As noted previously^{6,10}, the expression levels obtained are considerably lower (<10%) than those theoretically attainable. It has been suggested that this is due to inefficient transcription, inefficient translation and proteolytic degradation of eukaryotic molecules⁶.

The HA gene expressed in *E. coli* does not originate from an influenza strain which is currently causing disease. However, the methods described here are widely applicable and might be a basis for producing a vaccine against a current strain if the levels of expression can be increased.

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- Laver, W. G. & Kilbourne, E. D. *Virology* **30**, 493-501 (1966).
- Laver, W. G. & Webster, R. G. *Virology* **34**, 193-202 (1968).
- Laver, W. G. & Webster, R. G. *Virology* **48**, 445-455 (1972).
- Gething, M.-J., Bye, J., Skehel, J. & Waterfield, M. in *Structure and Variation in Influenza Virus* (eds Laver, W. G. & Air, G.) 1-10 (Elsevier, Amsterdam, 1980).
- Gething, M.-J., Bye, J., Skehel, J. & Waterfield, M. *Nature* **287**, 301-306 (1980).
- Fraser, R. H. & Bruce, B. J. *Proc. natn. Acad. Sci. U.S.A.* **75**, 5936-5940 (1978).
- Grunstein, M. & Hogness, D. S. *Proc. natn. Acad. Sci. U.S.A.* **72**, 3961-3965 (1975).
- Maxam, A. M. & Gilbert, W. *Meth. Enzym.* **65**, 499-560 (1980).
- Wiley, D. C., Wilson, L. A. & Skehel, J. J. *Nature* **289**, 373-378 (1981).
- Emtage, J. S. *et al. Nature* **283**, 171-174 (1980).
- Roberts, T. M., Bikel, I., Yogum, R. R., Livingston, D. M. & Ptashne, M. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5596-5600 (1979).
- Guarente, L., Lanes, G., Roberts, T. M. & Ptashne, M. *Cell* **20**, 543-553 (1980).
- Goeddel, D. V. *et al. Nature* **287**, 411-416 (1980).
- Goeddel, D. V. *et al. Nucleic Acids Res.* **8**, 4057-4074 (1980).
- Taniguchi, T. *et al. Proc. natn. Acad. Sci. U.S.A.* **77**, 5230-5233 (1980).
- Kupper, M. *et al. Nature* **289**, 555-559 (1981).
- Mandel, M. & Higa, A. J. *molec. Biol.* **53**, 159-162 (1970).
- Miller, J. H. *Experiments in Molecular Genetics* (Cold Spring Harbor Laboratory, New York, 1972).
- Birnboim, H. C. & Doly, J. *Nucleic Acids Res.* **7**, 1513-1523 (1979).
- Broome, S. & Gilbert, W. *Proc. natn. Acad. Sci. U.S.A.* **75**, 2746-2749 (1978).
- Ey, P. L., Prowse, S. J. & Jenkin, C. R. *Immunochimistry* **15**, 429-436 (1978).
- Fraker, P. J. & Speck, J. C. Jr *Biochem. biophys. Res. Commun.* **80**, 849-857 (1978).
- Weaver, R. F. & Weissmann, C. *Nucleic Acids Res.* **7**, 1175-1193 (1979).

Oestrogen receptor levels and vitellogenin synthesis during development of *Xenopus laevis*

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The synthesis of vitellogenin, the precursor of the major egg-yolk proteins, becomes inducible by oestrogen in *Xenopus laevis* larval liver during metamorphosis¹⁻⁴, well before vitellogenin is required for oocyte growth. The synthesis *in vivo* of some liver proteins is responsive to oestradiol at earlier stages, but vitellogenin inducibility does not appear until a precise point during metamorphic climax, stage 62, when many 'adult' liver proteins first start to be synthesized⁵. This suggests that vitellogenin inducibility is not determined simply by the presence of an oestrogen receptor. The receptor identified and characterized in fully inducible adult liver^{6,7} (also detected by others⁸) is unusual because it is present at very low concentrations and 50% is found in the nucleus in unstimulated animals^{6,7}, and is itself synthesized in response to oestrogen, raising the level in the nucleus⁹. Here we have measured nuclear receptor levels and the effect of oestrogen on these during *Xenopus* metamorphosis. Larvae which cannot be induced to synthesize vitellogenin contain an oestrogen receptor with the same affinity and specificity as the adult receptor. Treatment with oestrogen raises the level of nuclear oestrogen receptor in inducible but not in uninducible larvae. These and additional results from thyrostatic larvae suggest that an increase in the level of nuclear receptor is necessary for the induction of vitellogenin synthesis, and that the onset of vitellogenin inducibility might be determined by the ability of oestrogen to increase nuclear receptor levels.

We first measured receptor levels in salt extracts of liver nuclei from untreated and oestrogen-treated larvae at six different stages during metamorphosis, using methods⁶ which maximize the yield of soluble receptor from adult liver nuclei, but which would not extract any insoluble receptor. Figure 1 shows examples of the Scatchard plots of total ³H-oestradiol binding for four of the six stages examined. In all cases, the curved Scatchard plots could be resolved into two components¹⁰ with the high-affinity component having a dissociation constant close to 0.50×10^{-9} M, the value found previously⁶ for the oestrogen receptor in the adult. The relative amounts of low-affinity binding were estimated from the limiting bound/free values, which are proportional to the numbers of non-suppressible binding sites. The relative amounts of receptor and low-affinity binding were very different at different stages and the ratio was affected by oestrogen treatment. For example, the low-affinity component accounted for most of the binding in both untreated and oestrogen-treated stage 54 larvae, but for a much lower proportion in stage 66 animals and an even lower proportion in the same animals after oestrogen treatment. The larval livers were probably contaminated with plasma as it was not practicable to perfuse them; this could account for the often large amounts of low-affinity binding in the nuclear extracts. Neither progesterone, testosterone nor dexamethasone could bind to the larval receptor, and we conclude that the oestrogen receptors of larval and adult liver are indistinguishable by the criteria used here.

Figure 2 shows the number of receptor sites and the relative amounts of low-affinity binding present at various developmental stages. In untreated larvae the amount of receptor increases gradually from 49 sites per nucleus at stage 54 to 74 sites per nucleus at stage 66 (Fig. 2a). This compares with 100 sites per nucleus in the adult male⁶ and shows that there is, at the most, a twofold increase in the amount of nuclear receptor between stage 54 and the adult. In contrast, the amount of

low-affinity binding decreased about twofold between stage 54 and 3 months post metamorphosis (Fig. 2b). Vitellogenin synthesis is not inducible in larval liver before stage 62, but is clearly inducible at stage 62 during metamorphic climax, and inducibility increases during the completion of metamorphosis (stages 62-66) and the 3 months following it⁴. This study therefore establishes that the nuclear receptor is present in *Xenopus* liver before vitellogenin synthesis becomes inducible, and shows that the presence of the receptor is not by itself responsible for vitellogenin inducibility.

Figure 2a also shows the levels of nuclear receptor in oestrogen-treated larvae. In the three earliest stages, oestrogen had no effect on the nuclear receptor levels but from stage 62 onwards it progressively increased them, so that by 3 months after metamorphosis the increase in the nuclear receptor level was the same as that found in adult males after oestrogen treatment⁹. Oestradiol treatment did not alter the affinity of the receptor at any stage. The amount of low-affinity binding was

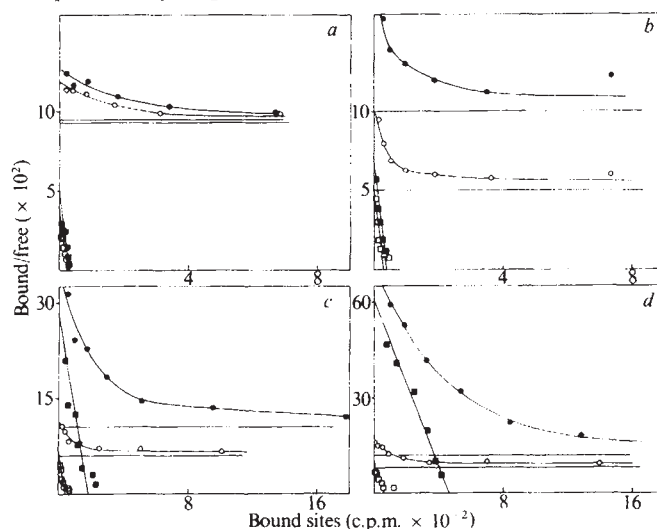


Fig. 1 Affinity of ³H-oestradiol binding in nuclear extracts of liver from untreated or oestrogen-treated *Xenopus* larvae. All procedures were performed at 0-4 °C. Livers were removed from larvae (staged according to Nieuwkoop and Faber¹³), pooled and washed with saline¹⁴. They were then weighed and 250 mg (equivalent to ~120 livers) were homogenized in 7 ml of homogenization buffer (0.25 M sucrose, 1 mM Tris-HCl, 1 mM MgCl₂, 150 µg ml⁻¹ phenylmethylsulphonylfluoride and 50 mM Bacitracin, pH 7.3) using six strokes of a Teflon-glass homogenizer. The homogenate was filtered through two layers of cheesecloth, washed through with another 4 ml of homogenization buffer and centrifuged at 800g for 10 min. The pellet was resuspended in 20 volumes of homogenization buffer and the number of hepatocyte nuclei, which were distinguishable from contaminating erythrocytes, was estimated by microscopic examination of an aliquot. The suspension was recentrifuged at 800g for 10 min and the pellet resuspended in extraction buffer (0.5 M KCl, 0.01 M Tris-HCl, 1 mM 2-mercaptoethanol, 10% glycerol, 150 µg ml⁻¹ phenylmethylsulphonylfluoride and 50 mM Bacitracin, pH 7.3) at 2 ml per g of original tissue, frozen and thawed, then homogenized in a small all-glass homogenizer. The nuclear extract was then centrifuged in a cooled Beckmann microfuge for 4 min and the supernatant stored in liquid nitrogen until use. Glass columns (9.5 × 0.4 cm diameter) were packed with Sephadex LH-20 which had been swollen in extraction buffer and then equilibrated at 4 °C. To remove endogenous oestrogen, 400 µl of the thawed extract were applied to the columns and eluted with 1 ml of extraction buffer. Aliquots of the eluates were incubated with steroid at 20 °C for 1 h to allow exchange of bound steroid with ³H-oestradiol⁹. 100 µl were applied to each column and eluted with 750 µl extraction buffer. Eluates were counted with 50% efficiency after extraction of radioactivity into scintillant (10 ml containing: PPO (diphenyloxazole), 4 g; POPOP (*p*-phenylenephenyloxazole), 0.1 g per litre of toluene). Equilibrium binding constants were estimated from the binding curves. We plotted the total binding to ³H-oestradiol according to Scatchard¹⁵ and determined the limiting bound/free ratio reached after the high-affinity component becomes saturated. This ratio was used to calculate the low-affinity binding at each free ligand concentration; this was subtracted from the total binding to give the binding due to the high-affinity component, which was then analysed according to Scatchard¹⁵ to give the *K_d* and number of binding sites. To study the effect of oestrogen, larvae were immersed for 3 days in 1 µM oestradiol in water, which was renewed daily, before staging them and removing their livers. The total ³H-oestradiol binding was determined at various concentrations of ³H-oestradiol in untreated (○) or oestrogen-treated (●) larvae and the high-affinity binding assayed in untreated (□) or oestrogen-treated (■) livers (as described above) at stage 54 (a), 57 (b), 62 (c) or 66 (d).

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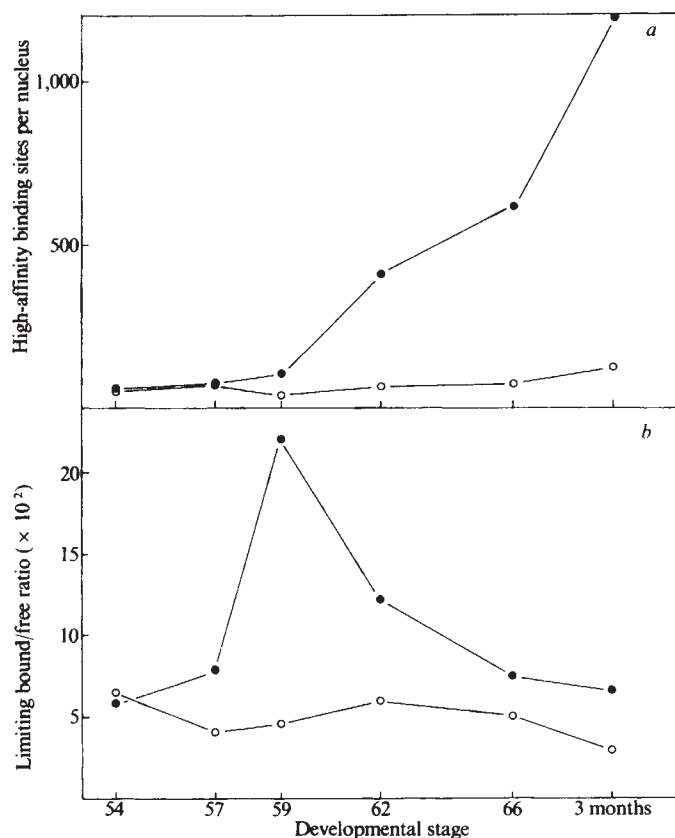


Fig. 2 Average number of high-affinity oestrogen-binding sites per nucleus (a) and the limiting bound/free ratio (b) as a measure of low-affinity binding in *Xenopus* liver during metamorphosis. At least two measurements were made at each stage. ○, Untreated and ●, oestrogen-treated larvae.

also increased by oestrogen treatment, but here the increase was greatest at the onset of metamorphic climax (stage 59) and much lower at later stages, being only twofold at stage 66. In contrast, the amount of nuclear oestrogen receptor was first increased by oestrogen treatment at stage 62, when vitellogenin synthesis becomes inducible. This suggests that the ability of oestrogen to induce vitellogenin synthesis at a certain stage during development is closely connected with its ability to increase receptor levels. We examined this relationship in further experiments.

An important advantage of developmental studies on *Xenopus* is that larval development can be arrested in late pre-metamorphosis using compounds such as propylthiouracil (PTU). As these animals age, events which would occur if they were developing normally will take place only if they do not depend on metamorphic development. This has allowed us to show⁵ that only half the alterations in liver protein synthesis normally associated with metamorphosis are dependent on developmental stage rather than on chronological age. If two events which occur during development retain the same temporal association in developmentally arrested animals, additional evidence is provided that one event requires the occurrence of the other. We therefore measured the level of oestrogen receptor and the effect of oestrogen on this level in liver from developmentally arrested larvae. The animals were first kept for 1 yr in water containing $10 \mu\text{g ml}^{-1}$ (0.58 mM) PTU, which was changed every 2 weeks. This arrested development at stage 54, but did not inhibit growth, so that at the time of use the thyrostatic larvae were considerably larger than normal larvae of the same developmental stage. Vitellogenin synthesis was uninducible in the stage 54 thyrostatic animals, but Fig. 3 shows that there was as much receptor (100 sites per nucleus) in the livers of these larvae as in adult males, confirming that receptor alone cannot confer inducibility. Figure 3 also shows that the level could not be increased by oestrogen treatment, so that in this respect the thyrostatic larvae at stage 54 behaved like normal larvae of the same stage. The amount of low-affinity binding was somewhat higher in arrested larvae and

was increased about twofold by oestrogen treatment whereas in normal larvae oestrogen had no effect on the amount of low-affinity binding.

Animals were then removed from PTU and allowed to metamorphose, and the nuclear oestrogen binding was measured at various stages during this delayed metamorphosis (Fig. 3), before and after treatment with oestrogen. Whereas the level of induction of the low-affinity binding decreased (Fig. 3b), the nuclear receptor levels were increased by oestrogen treatment from about stage 60 (Fig. 3a), the stage at which vitellogenin synthesis becomes inducible in these animals⁴. The increased level of the receptor in stage 62 animals which had previously been kept in PTU was about the same as in normal animals of a similar stage (Fig. 2a). In all cases the dissociation constant of the receptor found in larvae that had been treated with PTU was $0.50 \pm 0.05 \times 10^{-9}$ M, the value found for normal larvae.

These results show that while the normal, small rise in receptor content to the level found in adult males does not require metamorphosis, the ability of oestrogen to increase receptor levels does. In addition, in both normal and arrested animals oestrogen can first increase the level of nuclear receptor at the developmental stage when it can first induce vitellogenin synthesis. This precise correlation suggests that an increase in the level of nuclear oestrogen receptor is required for the induction of vitellogenin synthesis, and that the ability of oestrogen to increase the level of its receptor is in some way responsible for the appearance of vitellogenin inducibility. These data are consistent with results from other steroid-responsive systems^{11,12} where the rate of accumulation of the steroid-induced protein and its mRNA is thought to be determined by the level of nuclear receptor. In these systems the increase in nuclear receptor levels after hormone treatment is largely the result of translocation from a pre-existing pool of cytoplasmic receptor whereas in *Xenopus* liver it is probably due to synthesis of the receptor⁹. We have previously described⁵ a

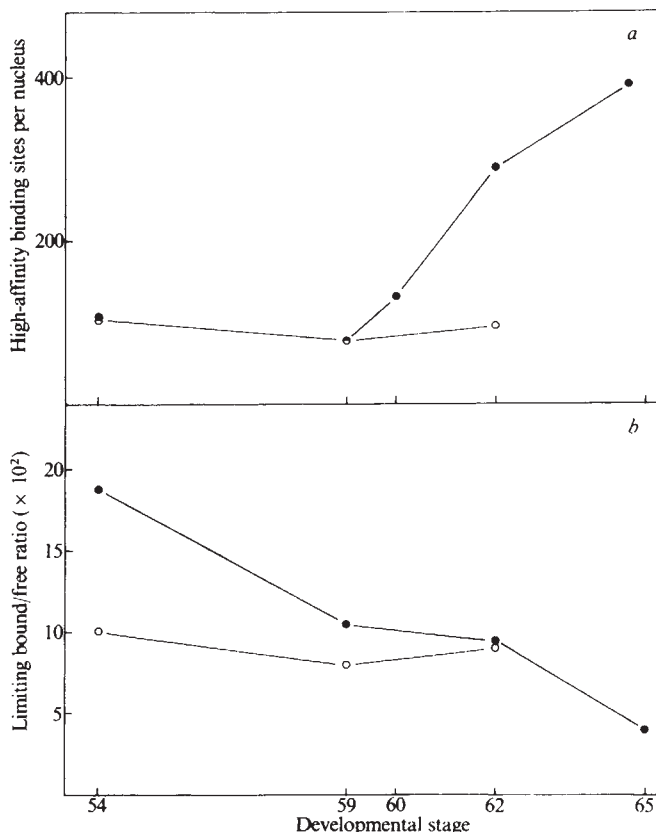


Fig. 3 Number of high-affinity oestrogen-binding sites per nucleus (a) and the limiting bound/free ratio (b) as a measure of the low-affinity binding in the livers of thyrostatic or previously thyrostatic *Xenopus* larvae. ○, Untreated and ●, oestrogen-treated larvae.

set of cellular proteins whose synthesis is influenced by oestrogen in larval liver before stage 62. Our data show that an elevated level of liver nuclear oestrogen receptor is not induced by oestrogen in animals of this stage. A model has been proposed^{11,12} in which different levels of nuclear oestrogen receptor are required to induce transcription of different genes, perhaps reflecting different affinities or numbers of nuclear acceptor sites, and our results are consistent with this type of model.

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1. Knowland, J. *Differentiation* **12**, 47–51 (1978).
2. Huber, S., Ryffel, G. U. & Weber, R. *Nature* **278**, 65–67 (1979).
3. Skipper, J. K. & Hamilton, T. H. *Science* **206**, 693–695 (1979).
4. May, F. E. B. & Knowland, J. *Dev Biol.* **77**, 419–430 (1980).
5. May, F. E. B. & Knowland, J. *Dev Biol.* **82**, 158–167 (1981).
6. Westley, B. R. & Knowland, J. *Cell* **15**, 367–374 (1978).
7. Westley, B. R. *Differentiation* **15**, 67–72 (1979).
8. Hayward, M. A., Mitchell, T. A. & Shapiro, D. J. *J. biol. Chem.* **255**, 11308–11312 (1980).
9. Westley, B. R. & Knowland, J. *Biochem. biophys. Res. Commun.* **88**, 1167–1172 (1979).
10. Chamness, G. C. & McGuire, W. L. *Steroids* **26**, 538–542 (1975).
11. Mulvihill, E. R. & Palmiter, R. D. *J. biol. Chem.* **252**, 2060–2068 (1977).
12. Mulvihill, E. R. & Palmiter, R. D. *J. biol. Chem.* **255**, 2085–2091 (1980).
13. Nieuwkoop, P. D. & Faber, J. *Normal Table of Xenopus laevis (Daudin)* 2nd edn (North Holland, Amsterdam, 1967).
14. Barth, L. G. & Barth, L. J. *J. Embryol. exp. Morph.* **7**, 210–222 (1959).
15. Scatchard, G. *Ann. N.Y. Acad. Sci.* **51**, 660–672 (1949).

Mycoplasmas induce collagenase in BALB/c 3T3 cells

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The turnover of collagen, the major protein of the body, is controlled by the rate of collagen synthesis and the activity of collagenase, a highly specific protease which initiates collagen degradation. Because collagen is largely resistant to other proteases, collagenase is believed to have an important regulatory role in such normal processes as tissue remodelling, wound healing and ageing. In addition, the regulation of the activity of collagenase may be altered in diseases of connective tissue, particularly rheumatoid arthritis, and in malignant invasion and metastasis. Collagenase is an extracellular enzyme, and thus it has been necessary to study its regulation in cells maintained in tissue culture, by measuring accumulation of the secreted protein in the culture medium. In this way, collagenases have been isolated from many tissues and cells^{1–7}. Little is known about the modulation of collagenase synthesis and secretion, but various treatments have been reported that induce its secretion in mammalian cells in monolayer culture^{8–17}, possibly by a mechanism involving membrane perturbation. We report here the production and secretion of collagenase by BALB/c 3T3 fibroblasts. Their ability to synthesize collagen^{18,19} has been extensively studied, but there has been no previous report of their collagenolytic activity. During studies of collagenase activity in BALB/c 3T3 cells, we noted that certain cultures had greatly increased enzyme activity corresponding to cultures in which contamination with mycoplasmas had been detected during routine assay. Here we present evidence that infection of cultures of BALB/c 3T3 cells with mycoplasmas results in the accumulation of high levels of collagenase in the medium.

Figure 1 shows the time course of accumulation of collagenase activity in the culture medium of BALB/c 3T3 cells known to be infected with mycoplasmas. Replicate cultures of BALB/c 3T3 cells were established and allowed to achieve confluence. Then the culture medium was changed and this point was taken as day 0 of culture. On each successive day from 1 to 9, the medium

from duplicate dishes was collected and assayed for collagenase activity as described in Fig. 1 legend. Collagenase activity began to accumulate in the medium at about day 2, increased rapidly for the next 2 days, and then began to decline slowly. We do not know whether this decrease in accumulated collagenase activity is due entirely to an increase in rate of inactivation that eventually exceeds the rate of synthesis, or whether the rates of synthesis and secretion also decline. No collagenase activity was detected in medium not treated with trypsin; all the collagenase secreted was therefore in an inactive form. In addition, cell homogenates had no detectable collagenase activity, either active or trypsin-activable, indicating that most or all of the collagenase synthesized by 3T3 cells is secreted into the medium. Cell numbers did not, within experimental error, change in the period of collagenase accumulation (during the first 4 days after medium change), in uninfected, newly infected or chronically mycoplasma-infected cultures. Cell densities in infected and uninfected cultures were similar at confluence and throughout the period of collagenase production.

The contaminating microorganism in the 3T3 cultures with high collagenolytic activity was identified as *Mycoplasma orale* on the basis of a growth inhibition test using dried antiserum-impregnated disks²⁰. The induction of collagenolytic activity in cultures of BALB/c 3T3 cells by infection with mycoplasmas was demonstrated by adding an aliquot of pure mycoplasma culture, obtained as described above, to uninfected cells, which had low but measurable activity. The time course of the appearance of collagenolytic activity in these newly infected cultures is shown in Fig. 2. In this experiment confluent cultures

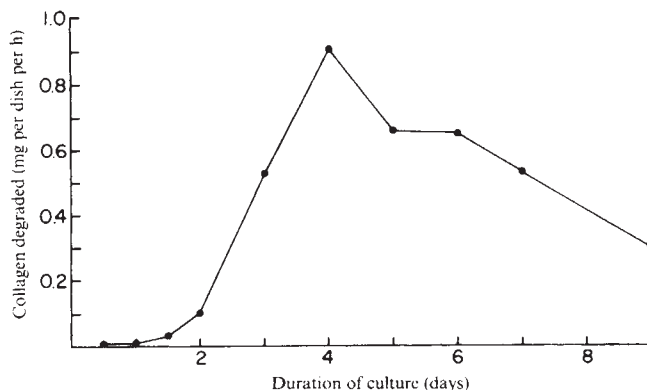


Fig. 1 Accumulation of collagenase activity in medium of BALB/c 3T3 cultures. BALB/c 3T3 cells were grown in Eagle's minimum essential medium (Gibco) with fourfold increased concentrations of vitamins and amino acids (Gibco) plus 10% fetal calf serum (Gibco), 100 U ml⁻¹ penicillin and 100 µg ml⁻¹ streptomycin (Sigma). The cells were plated into 10-cm plastic tissue culture dishes (Costar) containing 15 ml of medium. After the cells reached confluence, the medium in all dishes was replaced with fresh medium (day 0), and the cells maintained in these media for the time indicated. Media were collected from replicate cultures at the times indicated, centrifuged to remove debris, and brought to 0.1 M Tris-HCl, pH 7.5, and 0.05% sodium azide. Collagenase was precipitated from the conditioned medium by the addition of ammonium sulphate to 60% saturation. The precipitated protein was dissolved in, and dialysed against 0.05 M Tris-HCl, pH 7.5, 0.01 M CaCl₂, 0.05 M NaCl and 0.05% sodium azide. A 15-fold concentration of the starting volume was consistently achieved. All samples were then assayed for collagenase by measuring the release of soluble peptides from native collagen fibrils reconstituted at 37 °C (ref. 27). Each reaction mixture contained 36 µg of ¹⁴C-collagen in 10 µl of 0.1 M Tris-HCl, pH 7.5, 0.1 M NaCl, 0.01 M CaCl₂ and 0.05% sodium azide. The collagen had been radiolabelled by *in vitro* methylation to a specific activity of 667 c.p.m. per µg. A 30-µl aliquot of the collagenase preparation was added to the gelled collagen and treated with 30 µg of trypsin (Sigma no. T-1005) at room temperature for 45 min to activate the collagenase. A fourfold excess of soybean trypsin inhibitor (Sigma) was then added. Replicate reactions were incubated for increasing times at 37 °C and were terminated by centrifugation at 8,000g for 10 min. The entire supernatant fraction containing the digested collagen was counted in a liquid scintillation spectrometer. Blanks, which contained the same additions, including trypsin and trypsin inhibitor but no collagenase, were also run for each time of assay. Radioactivities released in these control reactions, which was generally less than 5% of the experimental (collagenase-containing) samples, were subtracted from the data shown. Units of collagenase activity were determined from the linear portion of each rate curve. Identical results were obtained in samples activated by dialysis against 1 mM APMA at 4 °C, in place of trypsin treatment.

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were established as described in Fig. 1 legend and the medium replaced with 15 ml of fresh medium to which was added 0.45 ml of a broth culture containing mycoplasmas. Control cultures received only fresh medium. Subsequently, the media were collected every 4 days and replaced by 15 ml of fresh medium. The media were assayed for collagenase activity as described in Fig. 1 legend.

As shown in Fig. 2, uninfected cells secreted relatively low levels of collagenase activity during each of the 4-day collection periods, whereas newly infected cells secreted increasing amounts of collagenase with time after infection. During the first 4 days, medium collected from both newly infected and control cultures had equivalent collagenase activity. However, during the second 4-day period, cultures infected with samples of mycoplasmas contained substantially increased collagenase activity. Medium from these infected cultures collected during the third 4-day period showed even higher collagenase activity, equal to that produced by cultures that had been infected before the start of the experiment (also shown in Fig. 2) and in which the level of collagenase activity had stabilized. In addition, parallel uninfected cultures received 0.45 ml of medium from infected 3T3 cultures instead of pure cultures of mycoplasmas. Collagenase activity in these cultures, also shown in Fig. 2, increased with a time course similar to that of the cultures treated with pure mycoplasmas. The increased level of collagenase activity in the third collection of medium from control cultures may be due to stimulation by accumulated collagen in the cultures; these cells are known to secrete and deposit collagen, which has been recently reported to stimulate the synthesis and secretion of collagenase⁸. On subculture, the level of collagenase in the control (uninfected) cell cultures dropped to the original level, while those in infected cultures remained high. The levels of collagenase that accumulate in culture medium exposed for 4 days to mycoplasma-infected cells are ~ 4.3 U per 10^6 cells (1 U = 1 μ g collagen degraded per min at 37 °C). Uninfected (control) 3T3 cells produce < 0.25 U per 10^6 cells. This is similar to the unstimulated/stimulated levels calculated from data reported for human skin fibroblasts exposed to collagen (0.36/7.14 U per 10^6 cells; see ref. 8), for rabbit corneal cells stimulated by lymphocytes from alkali-burned animals (0.7/6.1 U per 10^6 cells; ref. 16) and for human synovial fibroblasts stimulated by cytochalasin B (0.64–1.6/7.7–7.1 U per 10^6 cells; ref. 12).

The various cultures used in the experiment shown in Fig. 2, were re-examined for the presence of mycoplasmas. The control

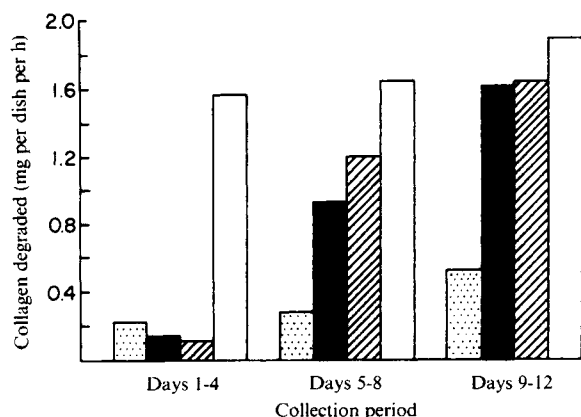
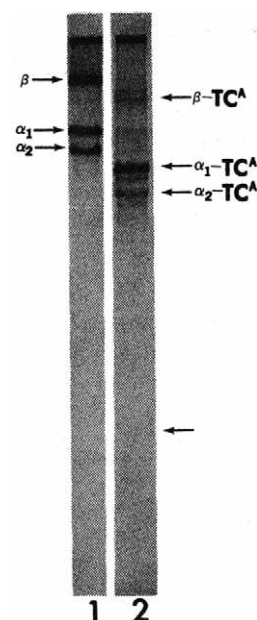


Fig. 2 Induction of collagenase activity in BALB/c 3T3 cultures after infection with mycoplasmas. BALB/c 3T3 cells were grown as described in Fig. 1 legend. After the cells reached confluence, the medium in all dishes was replaced with fresh medium (day 0). At this time, duplicate cultures received 0.45 ml of fresh medium (stippled bars); 0.45 ml of medium from infected BALB/c 3T3 cultures (solid bars); or 0.45 ml of a broth culture of mycoplasmas (cross-hatched bars). Parallel cultures of BALB/c 3T3 cells were grown in which mycoplasma infection had been previously established (open bars). Media were collected from each of these cultures every 4 days over a 12-day period and replaced with fresh media. After collection, the media were centrifuged to remove debris and brought to 0.05 M Tris-HCl, pH 7.5, and 0.05% sodium azide. Collagenase was measured as described in Fig. 1 legend.

Fig. 3 SDS-polyacrylamide gel electrophoresis of collagen after reaction with BALB/c 3T3 collagenase. Lane 1, 24 μ g of 14 C-collagen before incubation with collagenase. Lane 2, 24 μ g 14 C-collagen incubated with partially purified collagenase at 23 °C for 24 h. The reaction mixture contained 12 μ g of partially purified collagenase and 24 μ g of 14 C-collagen in a solution consisting of 0.05 M Tris-HCl, pH 7.5, 0.01 M CaCl₂, 0.05 M NaCl, 1 mM phenylmethylsulphonyl fluoride, 0.04% sodium azide and 6.25% glycerol in a final volume of 32 μ l. The collagenase used was purified by precipitation with (NH₄)₂SO₄ (40–60% saturation), batch elution from phosphocellulose, gradient elution from QAE-Sephadex A-50, gradient elution from phosphocellulose, and gel filtration on Sephadex G-100. The collagen had been radiolabelled by *in vitro* methylation²⁸ to a specific activity of 667 c.p.m. per μ g. The reaction was stopped by the addition of ice-cold 10% trichloroacetic acid. The protein was allowed to precipitate and then collected by centrifugation at 8,000g for 15 min. The protein pellet was rinsed twice with acetone, dried and dissolved in 20 μ l of electrophoresis sample buffer containing 10% glycerol, 0.1 M dithiothreitol, 2% SDS, 0.08 M Tris-HCl, pH 6.8, and 0.2% bromophenol blue. The sample was heated to 95 °C for 5 min then subjected to SDS-polyacrylamide gel electrophoresis (0.1% SDS in 12% polyacrylamide) according to the method of Laemmli²⁹. The gels were stained with Coomassie blue, dried and autoradiographed using Kodak X-Omat R film. The figure shows the autoradiograms of these gels. In lane 1, the positions of the α_1 , β -dimer, α_1 and α_2 are indicated. In lane 2, almost all the β -dimer, as well as most of the α_1 and α_2 polypeptides, have been digested. The positions of β -TC^A, the large cleavage product of β , and α_1 -TC^A and α_2 -TC^A, the large cleavage products of α_1 and α_2 , are indicated. The unlabelled arrow indicates the position of TC^B, which can be visualized by overexposing the autoradiogram or by incubating reaction mixtures with collagenase for shorter times.



cultures contained no detectable mycoplasmas, whereas the remaining three cultures, all of which showed increased collagenolytic activity, were infected. We have therefore concluded that infection of BALB/c 3T3 cells with mycoplasmas results in the increased accumulation of extracellular collagenase.

The collagenase produced by cultures of BALB/c 3T3 cells infected with *M. orale* seems to be a product of the murine 3T3 line, rather than that of the mycoplasma. We base this conclusion on the following evidence. (1) BALB/c 3T3 cells produce collagenase, although at a much lower level, before infection. (2) Mycoplasma organisms in broth, or transferred to culture medium in dishes containing no 3T3 cells, demonstrated no collagenase activity when assayed in the same conditions as BALB/c 3T3 culture medium. (3) We have partially purified the enzyme and found it to be similar to previously reported mammalian collagenases. Briefly, it degrades collagen at neutral pH; in crude preparations it requires treatment with trypsin or *p*-aminophenylmercuric acetate (APMA) for maximal activity; and it is completely inhibited by EGTA, orthophenanthroline and serum, partially inhibited by cysteine and dithiothreitol, and not inhibited by phenylmethylsulphonyl fluoride or soybean trypsin inhibitor. Most significantly, and in direct contrast to bacterial collagenases, collagenase purified from culture medium of mycoplasma-infected BALB/c 3T3 cleaves collagen into characteristic three-quarter length TC^A and one-quarter TC^B fragments, as shown in Fig. 3.

Thus, it seems that BALB/c 3T3 cells can synthesize and secrete relatively low levels of collagenase in normal circumstances. Infection of BALB/c 3T3 cells with mycoplasmas results in a significant increase in the accumulation of collagenase in the culture medium. Infected cultures of confluent cells show uniformly high levels of collagenase activity even after subculturing. In addition, the ability of mycoplasmas

to induce the synthesis and/or secretion of collagenase is not limited to BALB/c 3T3 cells. We have shown that cultures of chick fibroblasts also give dramatic increases in the level of extracellular collagenase activity on infection with mycoplasmas (data not shown). The induction of collagenolytic activity in cells by infection with mycoplasmas may have important clinical implications in terms of the development and persistence of connective tissue diseases. Mycoplasmas are recognized as agents possibly contributing to the establishment of rheumatoid arthritis in humans^{21,22} and are known to cause chronic arthritis

in certain animal species²³; increased levels of collagenase have been associated with joint destruction in rheumatoid^{24,25} and osteoarthritic²⁶ conditions. It seems reasonable to hypothesize that the involvement of mycoplasmas in disease states stems, at least in part, from their induction of collagenase.

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Received 13 March; accepted 23 June 1981.

- Harris, E. D. & Krane, S. M. *New Engl. J. Med.* **291**, 557-563, 603-609, 652-651 (1974).
- Stricklin, G. P., Bauer, E. A., Jeffrey, J. J. & Eisen, A. Z. *Biochemistry* **16**, 1607-1615 (1977).
- Woolley, D. E., Glanville, R. W., Roberts, D. R. & Evanson, J. M. *Biochem. J.* **169**, 265-276 (1978).
- Cawston, T. E. & Tyler, J. A. *Biochem. J.* **183**, 647-656 (1979).
- Woolley, D. E., Glanville, R. W., Crossley, M. J. & Evanson, J. M. *Eur. J. Biochem.* **54**, 611-622 (1975).
- Sakamoto, S., Sakamoto, M., Goldhaber, P. & Glimcher, M. J. *Archs Biochem. Biophys.* **188**, 438-449 (1978).
- Halme, J., Tyree, B. & Jeffery, J. J. *Archs Biochem. Biophys.* **199**, 51-60 (1980).
- Biswas, C. & Dayer, J.-M. *Cell* **18**, 1035-1041 (1979).
- Brinckerhoff, C. E. & Harris, E. D. *Excerpta med., Arthritis Rheum.* **21**, 745-753 (1978).
- Brinckerhoff, C. E., McMillan, R. M., Fahey, J. V. & Harris, E. D. *Excerpta med., Arthritis Rheum.* **22**, 1109-1116 (1979).
- Moscattelli, D., Jaffe, E. & Rifkin, D. B. *Cell* **20**, 343-351 (1980).
- Biswas, C. D., Reynolds, J. J. & Werb, Z. *Nature* **257**, 243-244 (1975).
- Wahl, L. M., Wahl, S. M., Mergenhagen, S. E. & Martin, G. R. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3598-3601 (1974).

- Wahl, L. M., Wahl, S. M., Sandberg, A. L. & Mergenhagen, S. E. *Proc. natn. Acad. Sci. U.S.A.* **74**, 4995-4958 (1977).
- Dayer, J.-M., Russell, R. G. & Krane, S. M. *Science* **195**, 181-183 (1977).
- Newsome, D. A. & Gross, J. *Cell* **16**, 895-900 (1979).
- Werb, Z. & Reynolds, J. J. *J. exp. Med.* **140**, 1482-1497 (1974).
- Green, H. & Goldberg, B. *Proc. natn. Acad. Sci. U.S.A.* **53**, 1360-1365 (1965).
- Peterkofsky, B. *Archs Biochem. Biophys.* **152**, 318-328 (1972).
- Stanbridge, E. & Hayflick, L. *J. Bact.* **93**, 1392-1396 (1967).
- Stanbridge, E. A. *Rev. Microbiol.* **30**, 169-187 (1976).
- Cassell, G. H. & Cole, B. C. *New Engl. J. Med.* **304**, 80-89 (1981).
- Washburn, L. R., Cole, B. C., Gelman, M. I. & Ward, J. R. *Excerpta med., Arthritis Rheum.* **23**, 825-836 (1980).
- Dayer, J.-M., Krane, S. M., Russell, G. G. & Robinson, D. R. *Proc. natn. Acad. Sci. U.S.A.* **73**, 945-949 (1976).
- Steere, A. C. *et al. Excerpta med., Arthritis Rheum.* **23**, 591-599 (1980).
- Erlich, M. G., Houle, P. A., Vigliani, G. & Mankin, H. J. *Excerpta med., Arthritis Rheum.* **21**, 761-765 (1978).
- Nagai, Y., Lapierre, C. M. & Gross, J. *Biochemistry* **5**, 3123-3130 (1966).
- Jentoft, N. & Dearborn, D. G. *J. biol. Chem.* **254**, 4359-4365 (1979).
- Laemmli, U. K. *Nature* **227**, 670-685 (1970).

Two distinct candidate transforming genes of lymphoid leukemia virus-induced neoplasms

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Avian lymphoid leukemia viruses (LLVs) are oncogenic retroviruses which induce tumours with relatively long latent periods and which seem to lack viral transforming genes¹. We previously reported that high molecular weight DNAs of seven LLV-induced tumours efficiently transformed NIH 3T3 mouse cells on transfection and that the transformed NIH cells did not contain viral DNA sequences detectable by hybridization with probes homologous either to the entire LLV genome or to the long terminal redundancy (LTR) of LLV DNA². These results suggested that oncogenesis by LLVs involved indirect activation of cellular transforming gene(s) which were not linked to LLV DNA. Hayward *et al.*³ recently found that 80-90% of LLV-induced metastatic bursal lymphomas contained exogenous LTR sequences near the cellular gene (*c-myc*) homologous to the presumptive transforming gene of myelocytomatosis virus strain MC29, a highly oncogenic avian acute leukaemia virus. Integration of LTR sequences containing the viral transcriptional promoter apparently increases transcription of *c-myc* in these lymphomas³. We now report that the LLV-induced tumours used as donors of DNA in our previous transfection experiments also contain LTR sequences near *c-myc*. However, further analysis of the NIH cells transformed by DNAs of LLV-induced bursal neoplasms indicates that transformation was not mediated by transfer of the tumour *c-myc* gene. These observations suggest that at least two different cellular genes with potential oncogenic activity are activated by different mechanisms in LLV-induced neoplasms.

DNAs of LLV-induced tumours were digested with *EcoRI*, electrophoresed in agarose gels, transferred to nitrocellulose filters and hybridized to ³²P-DNA probes for either the 5' LTR sequences of LLV DNA (p53-5' probe) or for the chicken *c-myc* gene (*pmc* probe, a gift of C. Pahl and M. Groudine) (Fig. 1).

³²P-DNA of *pmc* hybridized to two *EcoRI* fragments of normal chicken DNA with molecular sizes of ~14 and ~20 kilobases (kb) (Fig. 1). The 14-kb *EcoRI* fragment of normal chicken DNA is similar to the *c-myc*-containing *EcoRI* fragment⁴ and appears to contain all the chicken *c-myc* sequences. In addition to the 14- and 20-kb *EcoRI* fragments of normal chicken DNA, ³²P-DNA of *pmc* hybridized to a tumour-specific *EcoRI* fragment in DNAs of two neoplastic bursal nodules (T^c and T^d 2993), a metastatic bursal lymphoma (2902) and a nephroblastoma (3000) (Fig. 1). In each case, the tumour-specific *c-myc*-containing *EcoRI* fragment also hybridized to p53-5' probe (Fig. 1). Similar results were obtained with DNAs of a second metastatic lymphoma (B7362) and of a cell line derived from such a lymphoma (RP9) (not shown).

These observations are consistent with the findings of Hayward *et al.*³. As an *EcoRI* site is present within the LTR of exogenous LLVs^{5,6}, integration of an LTR near chicken *c-myc* in the tumour DNAs results in formation of a tumour-specific *EcoRI* fragment containing both 5' LTR and *c-myc* sequences.

EcoRI-digested DNAs of NIH cells² transformed by DNAs of the lymphoma cell line RP-9, metastatic lymphoma B7362, bursal nodule T^c 2993 and bursal nodule 2999 were hybridized to *pmc* probe to determine whether the transformed NIH cells contained *c-myc* sequences derived from the tumour DNAs (Fig. 2). The *c-myc*-containing *EcoRI* fragment of 14 kb was readily detectable in normal chicken DNA, which was included as a positive control. In contrast, *c-myc* sequences were not detected in NIH cells transformed by LLV-induced tumour DNAs. Although we have detected normal mouse sequences homologous to chicken *c-myc* as faintly hybridizing *EcoRI* fragments of ~10 kb in NIH 3T3 DNA, these bands are too faint for photographic reproduction. The absence of chicken *c-myc* sequences in NIH cells transformed by LLV-induced tumour DNAs indicates that the transforming gene of these tumours detected by transfection is different from the *c-myc* gene activated in the tumours by integration of LTR sequences.

LLV-induced tumours thus appear to contain at least two different genes with potential oncogenic activity: a *c-myc* gene activated by integration of a viral LTR and a distinct cellular gene capable of efficiently transforming NIH 3T3 cells. As both genes are present in most or all LLV-induced tumours so far studied^{2,3}, including a nephroblastoma, premetastatic bursal nodules and metastatic bursal lymphomas, it would seem that both genes are important in LLV-induced oncogenesis.

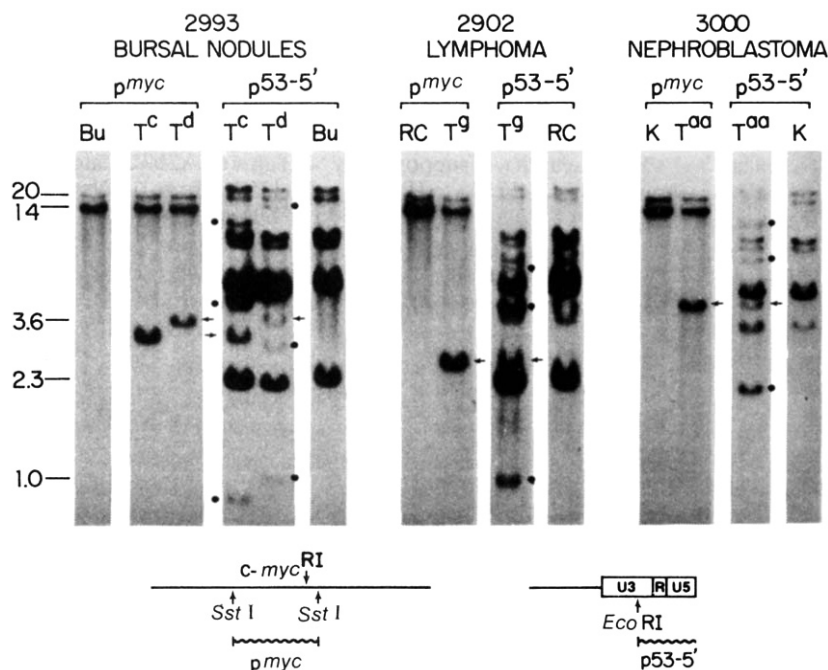


Fig. 1 Analysis of LLV-induced tumour DNAs. *EcoRI*-digested DNAs (10 µg) from two discrete bursal nodules (T^c and T^d) and surrounding normal bursa (Bu) from bird no. 2993, from a metastatic bursal lymphoma (T^g) and erythrocytes (RC) from bird no. 2902, and from a small nephroblastoma (T^{aa}) and normal kidney from bird 3000 were subjected to agarose gel electrophoresis and Southern blot hybridization using ^{32}P -DNA probes prepared by nick-translation of *pmyc* and *p53-5'*. *pmyc* contains a 3.2-kb *SstI* fragment which was subcloned in a derivative of pBR322 containing a single *SstI* site from a bacteriophage λ clone of *c-myc* isolated from the λ library of random chicken DNA fragments prepared by Dodgson *et al.*¹⁰. *p53-5'* was a fragment of *p53*¹¹ and contains the sequences (165 bp) from the *EcoRI* site in viral LTR to the terminus of linear LLV DNA. Tumour-specific fragments which contain both *c-myc* and *p53-5'* sequences are indicated by arrows. Additional tumour-specific fragments containing *p53-5'* sequences are indicated by circles. Molecular sizes (kb) are indicated for the normal chicken DNA fragments detected with *pmyc* probe (14 and 20 kb) and for the internal *EcoRI* fragments of LLV DNA detected previously¹² with probes representative of the entire LLV genome (1.0, 2.3 and 3.6 kb).

Activated cellular transforming genes which are not linked to viral DNA have also been detected by transfection of DNAs of mammary carcinomas induced by mouse mammary tumour virus which, like LLV, induces tumours with long latent periods and does not appear to contain a viral transforming gene⁷.

The pathogenesis of LLV-induced lymphomas suggests a multi-step process probably involving more than a single transformation event⁸. Activation of *c-myc* may therefore be involved in a stage of tumour development which precedes or complements activation of the transforming genes detected by transfection. Further studies will be required to define the roles of these two potential transforming genes in the disease.

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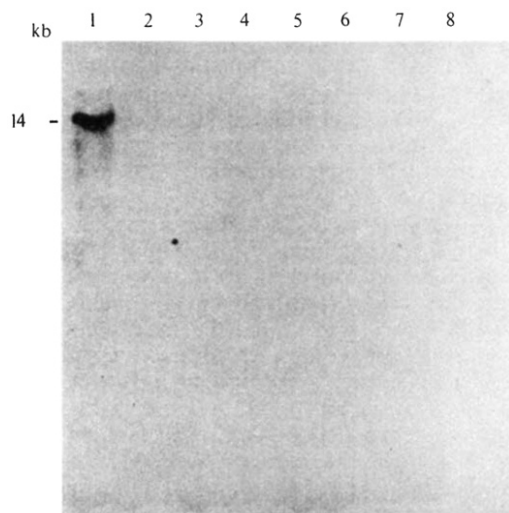


Fig. 2 Absence of chicken *c-myc* sequences in transformed NIH cells. *EcoRI*-digested DNAs (20 µg) of normal chicken embryo fibroblasts (lane 1), NIH 3T3 cells (lane 2), two independent lines of NIH cells transformed by DNA of bursal nodule T^d 2993 (lanes 3 and 4), two independent lines of NIH cells transformed by DNA of bursal nodule 2999 (lanes 5 and 6), NIH cells transformed by DNA of metastatic bursal lymphoma B7362 (lane 7) and NIH cells transformed by DNA of lymphoma cell line RP9 (lane 8) were analysed by blot hybridization with *pmyc* probe.

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1. Hanafusa, H. *Compreh. Virol.* **10**, 401-483 (1977).
2. Cooper, G. M. & Neiman, P. E. *Nature* **287**, 656-659 (1980).
3. Hayward, W. S., Neel, B. & Astrin, S. M. *Nature* **290**, 475-480 (1981).
4. Sheiness, D. K., Hughes, S. H., Varmus, H. E., Stubblefield, E. & Bishop, J. M. *Virology* **105**, 415-424 (1980).
5. Hsu, T. W., Sabran, J. L., Marks, G. E., Guntaka, R. V. & Taylor, J. M. *J. Virol.* **28**, 810-818 (1978).
6. Shank, P. R. *et al.* *Cell* **15**, 1383-1395 (1978).
7. Lane, M. A., Sauten, A. & Cooper, G. M. *Proc. natn. Acad. Sci. U.S.A.* **78** (in the press).
8. Neiman, P. E., Payne, L. N., Jordan, L. & Weiss, R. A. *Cold Spring Harb. Conf. Cell Proliferation* **7**, 519-528 (1980).
9. Southern, E. M. *J. molec. Biol.* **98**, 503-517 (1975).
10. Dodgson, J. B., Strommer, J. & Engel, J. D. *Cell* **17**, 879-887 (1979).
11. Neiman, P., Beemon, K. & Luce, J. A. *Proc. natn. Acad. Sci. U.S.A.* **78**, 1896-1900 (1981).
12. Neiman, P. E., Payne, L. N. & Weiss, R. A. *J. Virol.* **34**, 178-186 (1980).

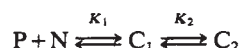
A tryptophan-containing peptide recognizes and cleaves DNA at apurinic sites

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Oligopeptides containing aromatic amino acids can preferentially form stacked complexes with single-stranded nucleic acids^{1,2}. Moreover, the peptide lysyl-tryptophyl- α -lysine (Lys-Trp-Lys) binds efficiently to locally destabilized regions in DNA after UV irradiation^{3,4} or modification by *N*-acetoxy-*N*(2)-acetylaminofluorene⁵. Lys-Trp-Lys also photosensitizes the cleavage of pyrimidine dimers in DNA³. We have recently shown that removal of purines in DNA introduces strong binding sites for this tripeptide through very efficient stacking of the tryptophyl residue with nucleic acid bases at apurinic sites⁶. We report here that incubation of partly depurinated DNA with Lys-Trp-Lys results in a specific cleavage of the DNA backbone probably due to the presence of the amino groups brought by the peptide into close proximity with the apurinic sites. This could provide a model for the enzymatic activity of AP endonucleases which are involved in the first step of the *in vivo* repair of apurinic site-containing DNA^{7,8}.

The binding of the peptide Lys-Trp-Lys (P) to nucleic acids (N) involves the formation of two complexes C_1 and C_2 according to a two-step model proposed earlier¹:



The tryptophyl ring of the peptide is stacked with nucleic acid bases in complex C_2 whereas only electrostatic interactions occur in complex C_1 . With a DNA containing apurinic sites the value of K_2 , which measures the ratio of the concentrations of stacked and unstacked complexes, is much higher than for any form of DNA damage previously studied^{3,6}. The values of K_2 for a native and an apurinic site are 0.3 and ≈ 200 , respectively⁶. Because of the high value of K_2 and the unmodified value of K_1 (ref. 6), the overall association constant [$K_1(1 + K_2)$] is more than two orders of magnitude higher for an apurinic site than for a native site. The peptide Lys-Trp-Lys can therefore recognize apurinic sites in a double-stranded structure through stacking interactions. The tryptophyl ring occupies the vacant site left by the removal of a purine⁶.

To test the ability of Lys-Trp-Lys to cleave the DNA backbone at apurinic sites, an average of two purines were removed from supercoiled covalently closed PM₂ DNA molecules (see Fig. 1 legend and ref. 9). Cleavage of one strand per DNA molecule converts the supercoiled DNA (form I) into the relaxed form (form II). As the electrophoretic mobilities of these two forms are different, agarose gel electrophoresis can be used to visualize single-strand breaks. Incubation of PM₂ DNA containing two apurinic sites in the presence of Lys-Trp-Lys resulted in a decrease in the intensity of the band corresponding to form I and a simultaneous increase in the intensity of the band corresponding to form II (results not shown). This effect was not

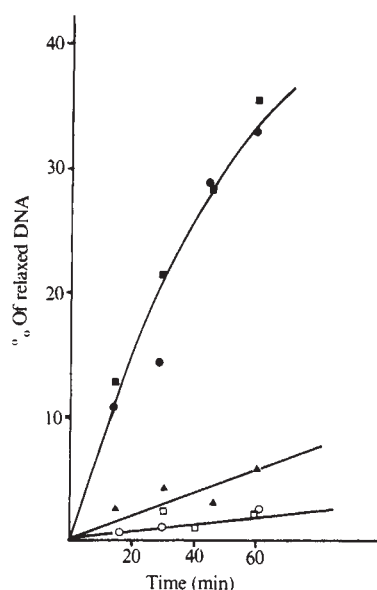


Fig. 1 Kinetics of single-strand break production in closed supercoiled PM₂ DNA containing two apurinic sites on incubation at 37 °C with Lys-Trp-Lys (●), Lys-Tyr-Lys (■), Lys-Gly-Lys (▲) or alone in the same conditions (control, ○). PM₂ DNA without apurinic sites was also incubated with Lys-Trp-Lys (□). PM₂ DNA containing 70% supercoiled molecules was incubated for 8 min at 70 °C in 0.1 M NaCl, 10 mM phosphate, 10 mM citrate (pH 5) to introduce about two apurinic sites per molecule⁹. These DNA samples were precipitated with ethanol and redissolved in 1 mM NaCl, 0.2 mM EDTA and 1 mM sodium cacodylate (pH 6). 10 µl of 10⁻⁴ M peptide solutions were added to 10 µl of 2 × 10⁻⁴ M DNA substrate at 37 °C. After the indicated time the solutions were withdrawn and diluted with 6 µl of a solution containing 50% glycerol and 0.1% bromophenol blue. The mixtures were loaded onto 0.8% agarose gels and the gels stained with ethidium bromide. Agarose gels were photographed and negatives were scanned with a spectrodensitometer. The ratio of relaxed and supercoiled DNA was estimated from the areas under the corresponding peaks.

observed when native DNA without apurinic sites was incubated with the peptide, indicating that the cleavage is related to the presence of apurinic sites in DNA. The amount of relaxed DNA molecules increased with the time of incubation: 35% of supercoiled DNA was cleaved after 60 min at 37 °C (Fig. 1). In the absence of Lys-Trp-Lys, incubation at 37 °C had a negligible effect on DNA containing apurinic sites (Fig. 1), whereas 1 h incubation of apurinic DNA with the peptide Lys-Gly-Lys resulted in the conversion of only 6% supercoiled DNA containing two apurinic sites to open DNA. The endonucleolytic activity is therefore clearly related to the specificity conferred to Lys-Trp-Lys by stacking interactions between the indole ring and nucleic acid bases, with the indole ring replacing the missing purine⁶. A tyrosine-containing peptide, Lys-Tyr-Lys, exhibited the same catalytic activity as Lys-Trp-Lys (Fig. 1), providing indirect evidence that the tyrosyl residue does stack with nucleic acid bases at apurinic sites.

Curves similar to those shown in Fig. 1 were obtained using fluorescence emission of intercalated ethidium bromide as a probe¹⁰. Intercalation of ethidium bromide molecules is favoured in relaxed relative to supercoiled DNA¹⁰. The ethidium bromide fluorescence emission was higher for apurinic DNA samples incubated with Lys-Trp-Lys than for DNA alone or for DNA incubated in the presence of Lys-Gly-Lys (results not shown). The time dependence of ethidium bromide fluorescence enhancement was similar to that of cleavage measured by gel electrophoresis.

The presence of an aromatic amino acid residue brings amino groups of lysyl residues into the vicinity of apurinic sites. At an apurinic site in DNA, the deoxyribose residue occurs in equilibrium between the free aldehyde and the furanose forms¹¹, and the previously reported effects of amines on the rate of chain cleavage probably depend on an interaction with these aldehydic groups¹². The present data suggest that the specific binding of Lys-Trp-Lys to apurinic sites enhances the local amine concentration. The different amines investigated by Lindahl and Andersson which promoted chain breakage at apurinic sites in DNA were added at 10⁻⁴-fold higher concentrations than those used with Lys-Trp-Lys¹².

The mechanism of Lys-Trp-Lys-induced strand breakage is unknown. Cleavage could involve the formation of a Schiff base between the aldehydic group of the ribosyl moiety at apurinic sites and an amino group of the peptide and/or a β-elimination reaction, as has been proposed for the cross-linking of histones to DNA after methylation followed by depurination¹³. To locate the NH₂ group involved in cleavage we are now investigating the activity of peptides in which the indole ring is separated from the potential 'active site' by glycyl residues (results to be reported elsewhere).

Our results show that the peptide Lys-Trp-Lys mimics both the damage specificity and the catalytic activity of endonucleases specific for apurinic sites (AP endonucleases). Stacking interactions provide a simple tripeptide with the essential recognition specificity required for this activity.

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1. Brun, F., Toulmé, J. J. & Hélène, C. *Biochemistry* **14**, 558–563 (1975).
2. Mayer, R., Toulmé, F., Montenay-Garestier, T. & Hélène, C. *J. Biol. Chem.* **254**, 75–82 (1979).
3. Toulmé, J. J., Charlier, M. & Hélène, C. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3488–3493 (1974).
4. Toulmé, J. J. & Hélène, C. *J. Biol. Chem.* **252**, 244–249 (1977).
5. Toulmé, F., Hélène, C., Fuchs, R. & Daune, M. *Biochemistry* **19**, 870–875 (1980).
6. Behmoaras, T., Toulmé, J. J. & Hélène, C. *Proc. natn. Acad. Sci. U.S.A.* **78**, 926–930 (1981).
7. Laval, J. *Nature* **269**, 829–832 (1977).
8. Verly, W. G. & Rassart, E. *J. Biol. Chem.* **250**, 8214–8219 (1975).
9. Lindahl, T. & Nyberg, B. *Biochemistry* **11**, 3610–3618 (1972).
10. Paolletti, C., Le Pecq, J. B. & Lehman I. R. *J. molec. Biol.* **55**, 75–100 (1971).
11. Overend, W. G. *J. chem. Soc.*, 2769 (1950).
12. Lindahl, T. & Andersson, A. *Biochemistry* **11**, 3618–3623 (1972).
13. Mirsabekov, A. D., Shick, V. V., Belyavsky, A. V. & Bavykin, S. G. *Proc. natn. Acad. Sci. U.S.A.* **75**, 4184–4188 (1978).

Sequence specificity of methylation in higher plant DNA

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Although plant DNA has a high content of 5-methylcytosine (5mC) very little is known about the distribution of this modification. Many of these methylations are found in the dinucleotide sequence C-G (refs 2-5) which is also the major modified site in animal cell DNA. It is clear, however, that C-G methylation cannot account for all the 5mC in DNA, in which this modified base may represent over 30% of the cytosine residues (as in certain varieties of plant¹). It was this observation that prompted the search for other methylated sites in the DNA. The results presented here show that methylated cytosine is indeed present at a variety of cytosine-containing dinucleotides, all of which, however, are part of the basic trinucleotide C-X-G. This sequence is probably essential for modification as it provides the symmetrical cytosines necessary for ensuring the inheritance of methylation at these sites.

The first step in characterizing plant DNA methylation was to determine the dinucleotide distribution of all the 5mC present in this organism. This was achieved by a modification of the standard nearest neighbour analysis which allows the detection of 5mC⁶. Plant DNA contains 5mC at C-A, C-T and C-C as well as C-G sites and the extent of methylation at these sequences is summarized in Table 1 (all sequences are in the 5' → 3' direction). In lieu of the nearest neighbour frequencies for this DNA⁷, the methylation observed in Table 1 accounts for the total methylcytosine of wheat-germ DNA, ~23-29% of all cytosines¹. Similar results were obtained for 2-week seedling wheat DNA and for other higher plant varieties, including tobacco.

Studies on the inheritance of 5mC in animal cells strongly suggest that the symmetrical presence of 5mC on both strands of the DNA is necessary to ensure the inheritance of this modification, at least at the sequence C-G. Because none of the dinucleotides C-A, C-T or C-C can accommodate this type of symmetry, we postulated that methylation at these sites might be based on a trinucleotide symmetry at the sequence C-X-G. This hypothesis is supported by the nearest-neighbour analyses for C-A and C-T (see Table 1), as it predicts that these dinucleotides will be found in the sequence $\begin{matrix} \text{C-A-G} \\ \text{G-T-C} \end{matrix}$ and would therefore be

methylated to the same extent. To examine this possibility, we developed an extension of the nearest-neighbour analysis technique which allows the detection of methylation at specific cytosine containing trinucleotide sequences. As shown in Table 1, over 80% of the trinucleotide sequences C-T-G and C-A-G were found to be modified, whereas the non-symmetrical sequence C-A-T was less than 4% methylated.

These partial sequence data strongly suggest that most, if not all, of the methylated C-A and C-T sequences in plant DNA are found adjacent to G in the prototype symmetrical trinucleotide C-A-G G-T-C. To validate this observation, we searched for restriction

enzymes which might recognize this site and be sensitive to cytosine methylation. Several restriction enzymes have recognition sequences which contain the sequence C-G but do not cleave when this site is methylated, for example, *HpaII*, *HhaI* and *AvaI* which have been used to analyse the methylation pattern of total mammalian DNA as well as specific genes^{8,9}. As expected, both *HpaII* and *HhaI* cleave plant DNA very poorly (data not shown). Individual restriction enzymes may be useful for detecting methylation at other sites. *EcoRII*, and *PstI* have

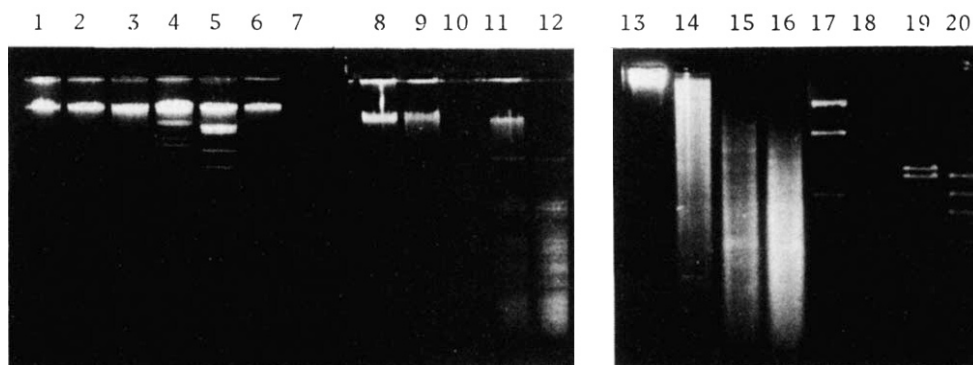
recognition sequences containing the trinucleotide sequence C-A-G G-T-C, but do not cleave when cytosine is methylated¹⁰. As shown in Fig. 1, wheat-germ and tobacco DNA are very poorly digested by these three enzymes, all of which digest animal DNA as expected. To determine the extent of methylation at these and other restriction sites, it was necessary to quantitate the degree of DNA digestion. To this end, the average molecular size of the

Table 1 Nearest-neighbour analysis of 5mC in plant DNA

DNA sequence	% Methylation
C-G	82
C-A	19
C-T	19
C-C	7
C-A-G	>80
C-T-G	>80
C-A-T	<4

DNA from wheat-germ was prepared as follows. Frozen wheat germ was ground under liquid nitrogen by mortar and pestle. The powder was dissolved in a mixture containing 10 mM Tris-HCl pH 7.9, 0.4 M NaCl, 5 mM EDTA, 0.5% SDS and 100 $\mu\text{g ml}^{-1}$ proteinase K (Merck), incubated for 1 h at 37 °C, extracted three times with phenol followed by three extractions with chloroform/isoamyl alcohol (24:1 v/v) and ethanol precipitated. The DNA preparation was treated for 30 min with 50 $\mu\text{g ml}^{-1}$ RNase A and further extracted with phenol and chloroform/isoamyl alcohol. The plant DNA (5 μg) was randomly nicked by sonication and nick translated with *E. coli* polymerase I (Biolabs) in the presence of a single [α -³²P]-deoxynucleoside triphosphate (200-500 Ci mmol⁻¹, NEN)⁶. The [α -³²P]-labelled DNA was digested to deoxynucleoside 3' monophosphates using micrococcal nuclease and spleen phosphodiesterase and applied to thin layer cellulose sheets (Eastman Kodak). The nucleotides were separated by two-dimensional chromatography and analysed by autoradiography as previously described⁶. Following TLC, the cytosine and 5-methylcytosine spots were scraped from the plastic sheet and the amount of radioactivity in each was determined by liquid scintillation counting. The percentage of cytosine methylation for each dinucleotide was then calculated $[(C/5mC + C) \times 100]$. In each case the nearest-neighbour frequencies of other dinucleotides appearing on the chromatogram were also measured to ensure that these correspond to published data on nearest-neighbour analyses. Partial trinucleotide sequencing was performed using the same basic technique modified to select specific trinucleotide sequences. DNA was nick translated in the presence of a single nucleotide as described above using either [α -³²P]dATP or [α -³²P]dTTP. Following a 5-min incubation at 15 °C, ddATP, ddCTP and ddTTP were added (6 mM each) and the reaction was continued for an additional 30 min. After this treatment only sequences containing Xp*TpG or Xp*ApG will have a free 3'-OH group. The DNA was isolated by phenol extraction, Sephadex column chromatography and ethanol precipitation. This DNA was denatured and those molecules containing a free 3'-OH end were elongated with poly(dA) tails using 18 units terminal transferase (PL Biochemicals). The specific molecules which contained the desired labelled trinucleotide sequence were then isolated by oligo(dT) cellulose chromatography²⁰. This DNA was then subjected to nearest-neighbour analysis⁹. Whereas ~20% of the C-A or C-T residues in plant DNA were found to be methylated, >80% of the trinucleotide sequence C-T-G and C-A-G were modified. In keeping with this, the DNA which did not stick to the oligo(dT) column and therefore did not contain poly(A) tails was only 5% methylated. This, of course, would include DNA containing all Cp*A and Cp*T residues which are not adjacent to G. For the C-A-T triplet, the procedure was modified slightly by using the dideoxynucleotides ddATP, ddGTP and ddCTP and the standard deoxynucleotide dTTP to select the correct sequence. The results are expressed as the percentage of methylation of the cytosine present in each sequence and should be considered as reliable estimates of the methylation content. The values shown for these triplets were obtained from the chromatogram and adjusted to take into account the instances where the second nucleotide is repeated. Thus, in addition to C-A-G the sequences C-A-A-G and C-A-A-A-G will also be selected by the oligo(dT) column and therefore contaminate the desired triplet. As indicated in Table 1, the values represent minimal estimates because these data have not been corrected for the fact that the dideoxynucleotide triphosphates are not 100% efficient even at the high concentration used in this experiment (unpublished results).

Fig. 1 Detection of plant DNA methylation by restriction enzyme analysis. DNA was prepared as described in Table 1 legend and digested by various restriction enzymes at a ratio of 2 units per μg DNA for 2 h using the buffers and conditions recommended by the suppliers. Restricted DNA was separated by electrophoresis on 1.5% (lanes 1–12) or 1.8% (lanes 13–20) agarose (Seakem) gels stained with ethidium bromide and photographed using Polaroid positive-negative type 665 film. Undigested tobacco DNA (lane 1) and undigested wheat-germ DNA (lanes 8 and 13) are shown for comparison. Tobacco DNA was cleaved with *Pst*I (lane 2), *Pvu*II (lane 3), *Eco*RII (lane 6) and *Bst*NI (lane 7). Tobacco DNA was also digested with *Pst*I (lane 4) and *Pvu*II (lane 5) in the presence of the marker λ phage DNA. Wheat-germ DNA was cleaved with *Eco*RII (lane 9), *Bst*NI (lane 10), *Msp*I (lane 14), *Hae*III (lane 15) and *Taq*I (lane 16). This DNA was also digested with *Eco*RII (lane 11) and *Bst*NI (lane 12) in the presence of the marker herpes simplex virus DNA. Lanes 17–20 contain molecular weight markers (250–4,500 bp) obtained by restriction digestion of various plasmid and phage DNAs.



DNA resulting from each restriction enzyme digestion was calculated from optical density scans of the ethidium bromide-stained agarose gels. The average experimental length of DNA obtained for each restriction enzyme can then be compared with the expected size computed on the basis of nearest-neighbour frequencies. For the enzymes *Hpa*II and *Hha*I the experimental length is 10 times greater than expected, indicating that this site is $\sim 90\%$ methylated in wheat-germ DNA. The restriction sites for *Pst*I, *Pvu*II and *Eco*RII were also found to be extensively methylated (Table 2). Note that the degree of methylation at *Pst*I and *Pvu*II sites represents an underestimate, because the average size of the DNA resulting from these digestions is probably higher, but not within the range of sensitivity for this particular gel. In the case of *Eco*RII the decreased digestion cannot be due to a lack of sites for this enzyme, because *Bst*NI, an isoschizomer of *Eco*RII that cleaves even if cytosine is methylated, cleaves this DNA normally (Fig. 1, Table 2).

In addition to methylation at C-A, C-T and C-G dinucleotides, we observed a small amount of methylation at C-C. It is tempting to suggest that these methylations are also localized in the trinucleotide site $\begin{smallmatrix} \text{C-C-G} \\ \text{G-G-C} \end{smallmatrix}$. One might expect that both

cytosines could be methylated in this sequence, as the internal cytosine is part of a C-G dinucleotide. The enzyme *Msp*I (C-C-G-G), which is inhibited by methylation at the external cytosine¹⁰, may be useful for probing methylation at C-C-G sites. The average expected molecular size of plant DNA after digestion with *Msp*I is 440 base pairs (bp). As shown in Fig. 1 and Table 2, the average size of DNA resulting from *Msp*I digestion is over twice that expected, suggesting that the external cytosine of this site is about 50% methylated.

*Bst*NI may be used to visualize directly the extent of methylation at $\begin{smallmatrix} \text{C-A-G} \\ \text{G-T-C} \end{smallmatrix}$ sequences. As this enzyme cleaves the

pentanucleotide sequence $\text{CC} \downarrow \begin{smallmatrix} \text{A} \\ \text{T} \end{smallmatrix} \text{GG}$ on the 3' side of the internal C, restriction digestion leaves DNA tails consisting of one nucleotide, A or T. These tails may be labelled with $[\alpha\text{-}^{32}\text{P}]\text{dATP}$ or $[\alpha\text{-}^{32}\text{P}]\text{dTTP}$ using the large subunit of *Escherichia coli* DNA polymerase I. When this DNA is subjected to nearest-neighbour analysis, the labelled phosphate is transferred to the internal C of the *Eco*RII sequence. If this cytosine is methylated, it should be revealed by chromatographic analysis of the 3'-monophosphate nucleotides. Autoradiography clearly shows that cytosine is the only labelled nucleoside phosphate, and that it is extensively methylated (90%) (Fig. 2). This experiment provides direct evidence that this particular site is highly methylated and suggests that other $\begin{smallmatrix} \text{C-A-G} \\ \text{G-T-C} \end{smallmatrix}$ sites are similarly modified. Because the cytosine residues adjacent to both A and T are 90% methylated, we also conclude that this site is symmetrically methylated on both DNA strands.

In animal cells 5mC accounts for about 2–7% of the total cytosine¹¹, whereas over 25% of the cytosine residues are methylated in plant cells¹. This difference is due to two major factors. Although in both plants and animals the C-G sequences are methylated to about 70–80%, the C-G dinucleotide is much more frequent in Plant DNA (3–4%) than animal DNA (0.5–1%)⁷. The second element which contributes to the high 5mC content of plants is methylation at other sites. Partial sequence analysis and restriction enzyme studies clearly show that almost all methylated C-A, C-C and C-T residues are found in the symmetrical trinucleotide sequence C-X-G.

It has previously been noted that C-G is a dinucleotide which contains symmetrical cytosine residues in both strands of the DNA and other evidence suggests that when this dinucleotide is modified both cytosines are indeed methylated^{9,12}. This symmetry is probably what enables the cell to transmit its methyl-

Table 2 DNA methylation at specific restriction enzyme sites

Source of DNA	Restriction enzyme	Expected size (bp)	Experimental size (bp)	% Methylation
Wheat germ	<i>Hpa</i> II (CCGG)	440	5,100	90
	<i>Eco</i> RII ($\begin{smallmatrix} \text{A} \\ \text{C} \end{smallmatrix} \text{CGG}$)	530	6,000	90
	<i>Bst</i> NI ($\begin{smallmatrix} \text{A} \\ \text{C} \end{smallmatrix} \text{TGG}$)	530	550	–
	<i>Pst</i> I (CTGCAG)	3,870	15,000	70
	<i>Pvu</i> II (CAGCTG)	3,870	14,000	70
	<i>Msp</i> I (CCGG)	440	920	50
	<i>Taq</i> I (TCGA)	380	330	–
	<i>Hae</i> III (GGCC)	330	390	–
	<i>Pst</i> I (CTGCAG)	3,250	3,900	–
	<i>Pvu</i> II (CAGCTG)	3,250	3,900	–
Mouse liver	<i>Pst</i> I (CTGCAG)	3,250	3,900	–
	<i>Pvu</i> II (CAGCTG)	3,250	3,900	–

Wheat-germ or mouse liver DNA was digested with various restriction enzymes at a ratio of 2 enzyme units per μg DNA for 2 h in the specific buffers recommended by the enzyme manufacturers (New England Biolabs, Bethesda Research Laboratories and Boehringer-Mannheim). The restricted DNA (5–10 μg) was subjected to gel electrophoresis on 0.6% agarose (*Hpa*II, *Eco*RII, *Bst*NI, *Pst*I, *Pvu*II), 1.5% agarose (*Msp*I, *Bst*NI) or 1.8% agarose (*Msp*I, *Taq*I, *Bst*NI, *Hae*III). Each gel was run together with at least 20 molecular length marker bands obtained by restriction enzyme digestion of various plasmid and phage molecules. Following electrophoresis, the ethidium bromide-stained gels were photographed using positive-negative Polaroid film and the film negative assayed using a scanning spectrophotometer. The resulting graphs were computer digitalized and the average molecular size calculated by reference to the molecular weight markers²⁰. These results were then corrected to take into consideration the size of the undigested DNA which was of the order of 50 kilobases. These results are accurate to about $\pm 10\%$. Expected average molecular size of each restriction enzyme was calculated from nearest-neighbour data⁷. The per cent methylation at each of these sites was determined by comparing the experimental and the expected size.

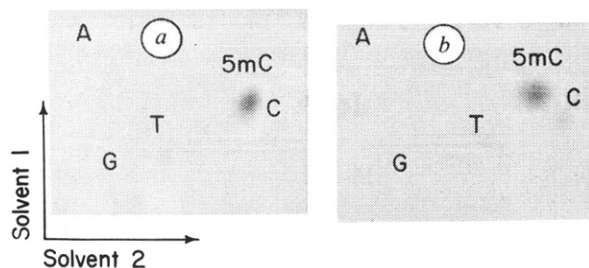


Fig. 2 The extent of methylation of the sequence CC^A_TGG in wheat-germ DNA. DNA (5 μ g), prepared as described in Table 1 legend, was cleaved with 10 units of *Bst*NI for 60 min at 60 °C. The DNA fragments were labelled at their 3' end by filling the sticky ends using *E. coli* DNA polymerase I (large fragment) with either [α - 32 P]dATP (a) or [α - 32 P]dTTP (b) for 10 min at 37 °C (ref. 21). The labelled fragments were digested to deoxynucleoside 3' monophosphates, chromatographed by two-dimensional TLC and autoradiographed⁶.

ation pattern to succeeding generations. In general, replicating DNA is in a state of hemimethylation since the parental strand is methylated at specific sites, which the newly replicated strand is as yet unmodified. The cellular 'maintenance methylase' would then act on the new strand using the parental strand methylation pattern as template^{11,13-15}. This model has received recent support from experiments in which unmethylated and *in vitro* methylated DNA were introduced into mouse L-cells by DNA-mediated gene transfer. Whereas unmethylated DNA remains unmodified, methylated foreign DNA faithfully inherits its *in vitro* acquired methylated pattern^{16,17}. We propose that the same symmetry that characterizes the dinucleotide C-G must be an integral part of any inheritable methylated residue. All the methylated sequences in plants seem to fit this criterion, as all methylations are either at the dinucleotide sequence C-G or the symmetrical trinucleotide sequence C-X-G.

The site $\begin{smallmatrix} C-C-G \\ G-G-C \end{smallmatrix}$ may be methylated at either of two different cytosines or at both. In either event, the single cytosine on the complementary strand would have to serve as a template for both cytosine methylations. The fact that this single methylated cytosine is part of a C-G residue suggests that in such cases the internal cytosine on the C-C-G strand will always be methylated, but the external cytosine may or may not be modified. This was indeed found to be the case for *Scilla* satellite DNAs analysed for methylation by DNA sequencing⁵. Methylation at the external cytosine of the trinucleotide C-C-G raises problems with regard to the inheritance of this methylation. As the complementary strand used as a template during replication contains only one methylated cytosine in the sequence C-C-G, it is difficult to understand how the methylase decides whether or not to methylate the external cytosine in addition to the internal cytosine. This decision may be random or determined by adjacent DNA sequences. Note that two specific C-C-G-G sites in the human globin gene battery were found to be resistant to *Msp*I and therefore methylated at the external cytosine in a tissue-specific manner^{18,19}. Because, in general, vertebrate DNA does not contain a detectable amount of C-C methylations, this type of animal DNA methylation is probably very rare.

As in vertebrate cells, the methylatable sites of plant DNA are not fully methylated. Approximately 80% of all C-G or $\begin{smallmatrix} C-A-G \\ G-T-C \end{smallmatrix}$ sites are methylated and only 50% of the C-C-G sites are modified at the external cytosine. It is this incomplete methylation which makes these sites potential factors in cellular regulation. A large body of evidence supports the view that DNA methylation may indeed play a part in the control of vertebrate gene expression. It is tempting to speculate that both the methylated C-G and C-X-G sites may also be involved in the regulation of gene expression in higher plants, but there is no evidence to support this. The enzymes *Pst*II, *Pvu*II and *Eco*RII

should prove useful for studying the relationship between gene expression and methylation in plant cells.

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- Shapiro, H. S. *CRC Handbk Biochem. molec. Biol.* **2**, 259 (1976).
- Bonen, L., Huh, T. Y. & Gray, T. W. *FEBS Lett.* **111**, 340-346 (1980).
- Burton, W. G., Grabow, C. T. & Sager, R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 1390-1394 (1979).
- Royer, H. D. & Sager, R. *Proc. natn. Acad. Sci. U.S.A.* **76**, 5794-5798 (1979).
- Deumling, B. *Proc. natn. Acad. Sci. U.S.A.* **78**, 338-342 (1981).
- Gruenbaum, Y., Stein, R., Cedar, H. & Razin, A. *FEBS Lett.* **124**, 67-71 (1981).
- Setlow, P. *CRC Handbk Biochem. molec. Biol.* **2**, 312-318 (1976).
- Bird, A. P. & Southern, E. M. *J. molec. Biol.* **118**, 27-48 (1978).
- Cedar, H., Solage, A., Glaser, G. & Razin, A. *Nucleic Acids Res.* **6**, 2125-2132 (1979).
- Gruenbaum, Y., Cedar, H. & Razin, A. *Nucleic Acids Res.* **9**, 2509-2515 (1981).
- Razin, A. & Riggs, A. D. *Science* **210**, 604-610 (1980).
- Bird, A. P. *J. molec. Biol.* **118**, 46-60 (1978).
- Riggs, A. D. *Cytogenet. cell. Genet.* **14**, 9-14 (1975).
- Holliday, R. & Pugh, J. E. *Science* **187**, 226-232 (1975).
- Razin, A. & Friedman, J. *Prog. Nucleic Acids Res. molec. Biol.* **25**, 33-52 (1981).
- Pollack, Y., Stein, R., Razin, A. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* **77**, 6463-6467 (1980).
- Wigler, M., Levy, D. & Perucho, M. *Cell* **24**, 33-40 (1981).
- van der Ploeg, L. H. T. & Flavell, R. A. *Cell* **19**, 947-958 (1980).
- van der Ploeg, L. H. T., Groffen, J. & Flavell, R. A. *Nucleic Acids Res.* **20**, 4563-4574 (1980).
- Naveh, T. & Cedar, H. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- Sneider, W. T. *Nucleic Acids Res.* **8**, 3829-3840 (1980).

Active multi-subunit ACh receptor assembled by translation of heterologous mRNA in *Xenopus* oocytes

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The acetylcholine receptor (AChR) mediates synaptic transmission on binding to the acetylcholine neurotransmitter. To gain further information on the composition and biosynthesis of this receptor (for review of properties see refs 1-3), we have extracted AChR mRNA and translated it in a cell-free system and also in *Xenopus* oocytes. The latter have been shown to be not only highly efficient translation systems for microinjected, heterologous mRNAs but they can also faithfully execute post-translational processes such as glycosylation, sequestration, pre-peptide cleavage and secretion of appropriate products⁴⁻⁷. As native AChR is found glycosylated and sequestered in the plasma membrane, this system is of potential interest in studying its biosynthesis. We report here that *Xenopus* oocytes efficiently assemble intact, multi-subunit AChR molecules which show properties characteristic of the native AChR, including the binding of α -bungarotoxin (α -BTX).

Initially, cell-free translation⁸ products of mRNA extracted from the electric organ of the ray, *Torpedo marmorata*, were immunoprecipitated with rabbit antiserum raised against the native AChR. AChR-specific polypeptides with apparent molecular weights (MWs) of 40,000 (40 K), 51 K and 59 K were clearly identified; two smaller and less abundant species were also found (Fig. 1). These sizes differ from those of the subunits thought to comprise native AChR¹⁻³; unlike the latter, the cell-free translation products were unable to bind α -BTX, which agrees with previous work⁹. Thus, the cell-free system seems to be incapable of faithfully synthesizing intact receptor molecules.

Experiments in which *Torpedo* mRNA was injected into *Xenopus* oocytes gave better results. Figure 2 shows that

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α -BTX binding activity could be detected in the translation products of the oocytes, the actual level of activity increasing with the amount of heterologous mRNA injected. To characterize the α -BTX binding component, ^{35}S -methionine-labelled translation products were purified by affinity chromatography on α -BTX-Sepharose (for details see Fig. 3 legend), and analysed by SDS-polyacrylamide gel electrophoresis¹⁰ (Fig. 3b, lane 1). As a control for nonspecific binding to the Sepharose, an aliquot of the extract was preincubated with a large excess of α -BTX before chromatography and electrophoresis (Fig. 3b,

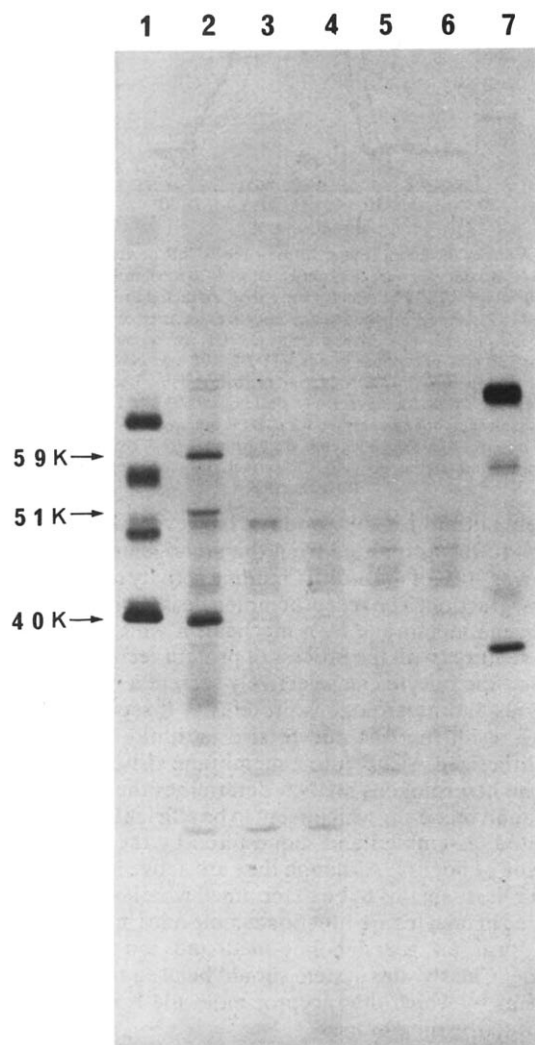


Fig. 1 Electrophoretic analysis of AChR polypeptides synthesized in a cell-free system. Poly(A) mRNA ($25 \mu\text{g ml}^{-1}$) extracted from the electric organ of *Torpedo marmorata*¹⁶ was translated in a nuclease-treated rabbit reticulocyte lysate⁸ in the presence of ^{35}S -methionine ($\sim 1 \text{ mCi ml}^{-1}$). The translation products were immunoprecipitated using formalin-fixed *Staphylococcus aureus* cells (SAC)¹³. The SAC antibody-antigen complexes were dissolved in SDS sample buffer¹⁰ and SAC removed by centrifugation at $10,000g$ for 1 min. The supernatants were electrophoresed through a 10% (w/v) SDS-polyacrylamide gel¹⁰ before fluorography¹⁷. Lane 1, AChR purified from *T. marmorata* electric organ by affinity chromatography then radioactively-labelled with succinimidyl [2,3- ^3H]propionate¹¹. This radiolabelling process gives the appearance of doublets after gel electrophoresis, which are absent in non-radioactive purified preparations of the native AChR (see Fig. 3a). Lane 2, immunoprecipitate obtained with antibody raised against native *Torpedo* AChR. Lane 3, immunoprecipitate obtained with antibody raised against *Torpedo* AChR but in the presence of a large excess of native *Torpedo* AChR; control for lane 2. Lane 4, immunoprecipitate obtained with normal rabbit serum; lane 5, immunoprecipitate produced with antibody against α -BTX (control for lane 6). Lane 6, immunoprecipitate obtained by pre-incubating with α -BTX, before addition of antibody raised against α -BTX. Lane 7, ^{125}I -labelled standard proteins—bovine serum albumin (68 K), catalase (58 K), ovalbumin (43 K) and lactate dehydrogenase (35 K). Titres of antibody raised against native *Torpedo* AChR and α -BTX were $\sim 2 \times 10^{-6} \text{ M}$ and $\sim 4 \times 10^{-6} \text{ M}$, respectively.

lane 2). Lanes 1 and 2 of Fig. 3b show four toxin-specific polypeptides of MWs 40 K, 49 K, 58 K and 66 K; these sizes are very similar to those of the native *Torpedo* AChR polypeptides that had been purified by α -toxin-gel affinity chromatography and then radioactively labelled¹¹ by ^3H -propionylation (Fig. 3b, lane 3). The native subunit sizes (Fig. 3a) were estimated to be 40 K, 49 K, 57 K and 65 K, as generally found for the α , β , γ and δ subunits^{2,3,12}, respectively, which comprise the *Torpedo* receptor molecule. A similar electrophoresis pattern was also obtained when oocyte translation products were purified by binding to α -BTX, followed by immunoprecipitation with α -BTX antibody and protein A¹³.

The size of the AChR molecule synthesized in oocytes was analysed by centrifugation through sucrose density gradients. As Fig. 4 shows, the AChR purified from the *Torpedo* electric organ exists in two molecular forms having sedimentation coefficients of 9S and 13S (monomer and dimer), an observation generally made¹⁻³ for *Torpedo* AChR preparations, the 9S form being predominant in reducing conditions. The monomeric 9S form has been shown^{2,3,12} to contain five subunits ($\alpha_2, \beta, \gamma, \delta$), corresponding to a MW of $\sim 250,000$. It was therefore interesting that on a parallel sucrose density gradient (Fig. 4), the translation products from the microinjected oocytes produced a peak of toxin-binding activity at the same position (9S) as the native monomer. This result clearly indicates the ability of the oocyte to assemble the newly synthesized subunits into intact receptor molecules. After sedimentation of ^{35}S -methionine-labelled translation products and immunoprecipitation of these gradient fractions with the antiserum raised against native AChR, SDS-gel electrophoresis showed that the receptor-specific peptides were present only in the 9S peak (data not shown). Assuming that this antibody can recognize newly synthesized, unassembled AChR polypeptides, which is the

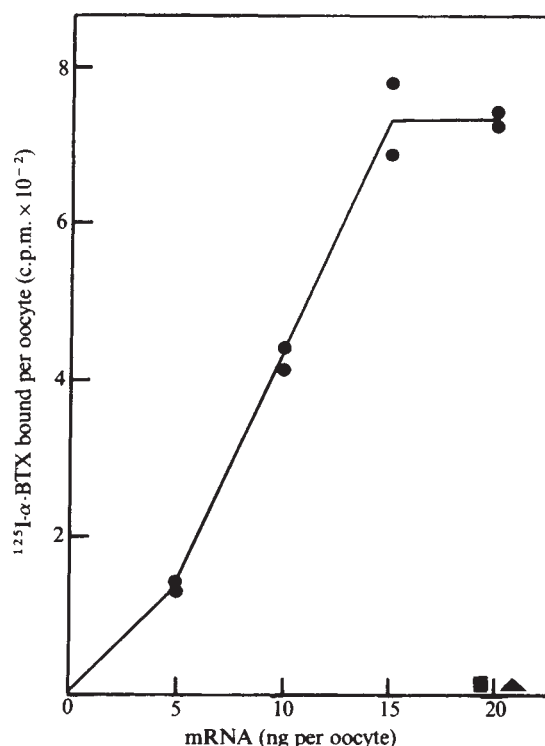


Fig. 2 α -BTX binding activity in *Xenopus* oocytes after microinjection with *Torpedo* mRNA. Duplicate batches of 15 oocytes were microinjected⁵ with various amounts of poly(A) mRNA that had been extracted¹⁶ from the electric organ of *T. marmorata* and cultured at 21°C in 150 μl modified Barth's medium⁵ for 48 h. The oocytes were then homogenized in 200 μl of 50 mM phosphate buffer, pH 7.2/1% Triton X-100/1 mM EDTA/1 mM EGTA/0.1 mM phenylmethylsulphonyl fluoride (PMSF). The ^{125}I - α -BTX¹⁸ binding activity ($\bullet$) in the resulting supernatants was measured¹⁹ after centrifugation at $10,000g$ for 30 min. $\blacktriangle$, Control in which a large excess of unlabelled α -BTX was added just before the assay. $\blacksquare$, As for experimental sample except that before the assay, the extract was incubated with Con A-Sepharose²⁰.

likely interpretation of the data from the cell-free system (see Fig. 1 and ref. 9), it seems that the assembly process in oocytes is efficient.

As in the case of the native *Torpedo* receptor^{12,14}, the AChR synthesized in microinjected oocytes is also glycosylated, as it binds completely to concanavalin A (ConA)-Sephacel (Fig. 2). The γ -subunit synthesized in oocytes may be slightly larger than that in the purified native AChR, thus it is possible that glycosylation and/or other processing events in the oocyte are not

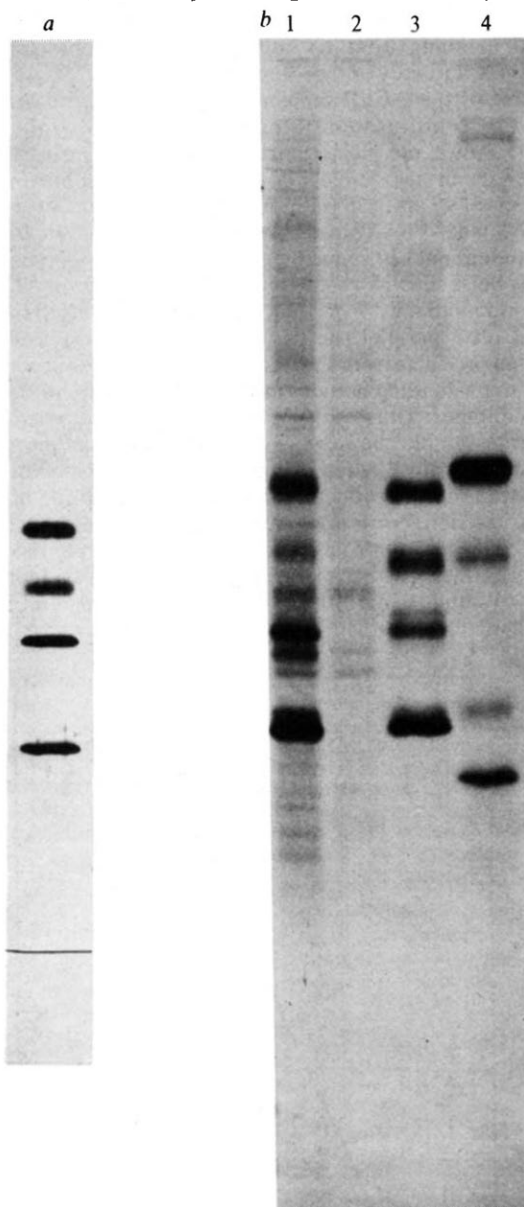


Fig. 3 Electrophoretic analysis of the AChR synthesized in oocytes as purified by affinity chromatography on α -BTX-Sepharose. *a*, A stained gel containing nonradioactive AChR purified from *T. marmorata*¹¹. (Note that this gel is shorter than that in *b*.) *b*, Batches of 40 oocytes were microinjected with *Torpedo* poly(A) mRNA (20 ng per oocyte) and cultured in 300 μ l Barths' medium containing 0.3 mCi ³⁵S-methionine at 21 °C for 48 h. They were then homogenized in 500 μ l of 50 mM phosphate buffer, pH 7.2/1% Triton X-100/1 mM EDTA/1 mM EGTA/5 μ g ml⁻¹ soybean trypsin inhibitor/1 mM benzamide/100 μ g ml⁻¹ bacitracin/0.1 mM PMSF, and centrifuged at 10,000g for 30 min. The supernatant was shaken with 0.1 ml α -BTX-Sepharose, which was then washed¹¹. All purifications were done as rapidly as possible in the cold and in the presence of protease inhibitors to limit proteolysis¹¹. Lane 1, AChR was eluted from the α -BTX-Sepharose using 50 μ l of 2% SDS in sample buffer¹⁰, and 25 μ l electrophoresed. Lane 2, a control preparation containing the same ³⁵S-labelled translation products, preincubated with a large excess of α -BTX before application to α -BTX-Sepharose. Lane 3, ³H-propionylated native *Torpedo* AChR (see Fig. 1). Lane 4, ¹²⁵I-labelled standard proteins (see Fig. 1). A 10% (w/v) SDS-polyacrylamide gel¹⁰ was used; *b* was subjected to fluorography¹⁷ and a to silver staining²¹.

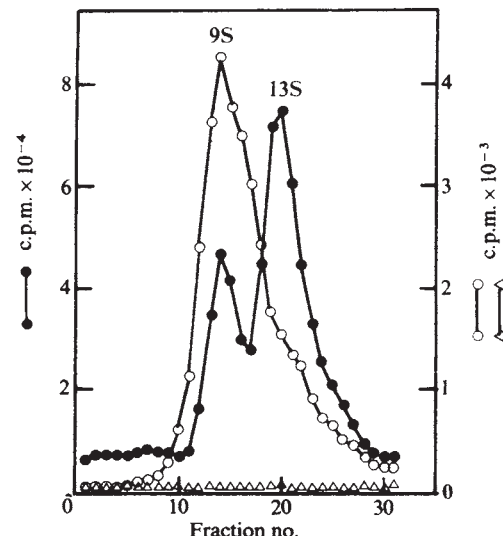


Fig. 4 Analysis of the molecular form of the AChR newly synthesized in oocytes. 40–50 oocytes were microinjected with 20 ng of mRNA per oocyte and cultured for 48 h. The oocytes were then extracted in 200 μ l buffer as described in Fig. 2 legend. Samples of oocyte extracts or of AChR purified from *T. marmorata* were loaded on to 5–20% (w/w) sucrose gradients containing 50 mM phosphate buffer, pH 7.2/100 mM NaCl/0.2% Triton X-100/1 mM EDTA/0.02% NaN₃ and centrifuged in a Beckman SW60 Ti rotor at 59,000 r.p.m. for 6 h (ref. 11). Fractions (0.13 ml) were collected by upward displacement and analysed for α -BTX binding activity using ¹²⁵I- α -BTX as described in Fig. 2 legend. ●, Purified AChR from *Torpedo*; ○, extract from uninjected oocytes; ○, extract from oocytes injected with *Torpedo* mRNA.

completely faithful for this product. However, when detergent (Triton X-100) was omitted from the homogenization medium (Fig. 2), over 90% of the α -BTX binding activity remained in the membrane fraction. The receptor molecule is transported *in vivo* to the plasma membrane by a mechanism which shares many common features with the process of protein secretion¹⁵. As it is known that the oocyte can selectively secrete a wide variety of homologous and heterologous proteins^{6,7}, it seems likely from the above result that the oocyte also faithfully sequesters the newly synthesized AChR into a membrane structure.

Thus the heterologous mRNA determines the production of multi-subunit receptors which seem to be efficiently synthesized, glycosylated, assembled and sequestered by the oocyte with a high degree of fidelity. Although they are active in that they can bind α -BTX, it remains to be determined whether the receptors synthesized in oocytes are functional molecules in terms of being able to form an acetylcholine-mediated ion channel in a membrane. Clearly, this system should be used to elucidate the mechanisms by which the receptor molecule is assembled and inserted into the membrane.

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- Heidmann, T. & Changeux, J. P. *Rev. Biochem.* **47**, 317–357 (1978).
- Karlin, A. in *Cell Surface Reviews* Vol. 6 (eds Poste, G., Nicolson, G. L. & Cotman, C. W.) 192–242 (North Holland, New York, 1980).
- Raftery, M. A., Hunkapiller, M. W., Strader, C. D. & Hood, L. E. *Science* **208**, 1454–1456 (1980).
- Gurdon, J. B., Lane, C. D., Woodland, H. R. & Marbaix, G. *Nature* **233**, 177–182 (1971).
- Gurdon, J. B. *The Control of Gene Expression in Animal Development* (Clarendon, Oxford, 1974).
- Lane, C. D., Shannon, S. & Craig, R. *Eur. J. Biochem.* **101**, 485–495 (1979).
- Lane, C. D. *et al. Eur. J. Biochem.* **111**, 225–235 (1980).
- Pelham, H. R. B. & Jackson, R. J. *Eur. J. Biochem.* **67**, 247–256 (1976).
- Mendez, B., Valenzuela, P., Martial, J. A. & Baxter, J. D. *Science* **209**, 695–697 (1980).
- Laemmli, U. K. *Nature* **277**, 680–685 (1970).
- Sumikawa, K., Barnard, E. A. & Dolly, J. O. *Eur. J. Biochem.* (submitted).
- Lindstrom, J., Merlie, J. & Yoo, G. *Biochemistry* **18**, 4465–4469 (1979).
- Ivarie, R. D. & Jones, P. P. *Analyt. Biochem.* **97**, 24–35 (1979).
- Vandlen, R. L., Wu, W. C. S., Eisenach, J. C. & Raftery, M. A. *Biochemistry* **18**, 1845–1854 (1979).
- Rotundo, R. L. & Fambrough, D. M. *Cell* **22**, 595–602 (1980).
- Houghton, M. *et al. Nucleic Acids Res.* **8**, 1913–1931 (1980).
- Laskey, R. A. & Mills, A. D. *Eur. J. Biochem.* **56**, 335–341 (1975).
- Vogel, Z., Sytkowski, A. J. & Nirenberg, M. W. *Proc. natn. Acad. Sci. U.S.A.* **69**, 3180–3184 (1972).
- Weinberg, C. B. & Hall, Z. W. *Proc. natn. Acad. Sci. U.S.A.* **76**, 504–508 (1979).
- Lyddiatt, A. *et al. FEBS Lett.* **108**, 20–24 (1979).
- Switzer, R. C., Merril, C. R. & Shifrin, S. *Analyt. Biochem.* **98**, 231–237 (1979).

MATTERS ARISING

The arrival of *Equus*

EVEN though papers which attempt to draw together a lot of diverse evidence are most important for the scientific community the authors of such papers should not neglect to acknowledge the basic analytical work on which they base their discussion. I feel that J. Brunet and I deserve to be quoted in discussion of the arrival of *Equus* in the Old World at least for Roccaneyra², probably the earliest European site to have yielded *Equus*, and probably the only one where *Equus* and *Hipparion* coexist. So far as I know, it was not V. J. Maglio³ but D. A. Hooijer⁴ and myself⁵ who, independently, stated that the first occurrence of *Equus* in the Omo beds was in member G of the Shungura Formation. Since 1973, we have often repeated that the arrival of *Equus* in Africa was about two million years ago⁴⁻⁹.

Lindsay *et al.*'s¹ bibliography is quite instructive. Most of the papers cited on the first occurrence of *Equus* in Europe and Africa are themselves reviews, rather than original papers describing new material or stating new facts. People like J. Brunet, who has worked for years with equids, or D. A. Hooijer and myself, who have published about 30 papers dealing with equids, are ignored, although we were responsible for the basic descriptions and determinations.

I am sure that any specialist whose colourless original work has been neglected, involuntarily or not, in more appealing papers will understand why I decided, even so late, to write about such a trifle.

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1. Lindsay, E. H., Opdyke, N. D. & Johnson, N. M. *Nature* **287**, 135-138 (1980).
2. Eisenmann, V. & Brunet, J. in *Int. Colloquium on the Problem 'The Boundary Between Neogene and Quaternary'* **4**, 104-122 (Moscow, 1973).
3. Maglio, V. J. *Nature* **239**, 379-385 (1972).
4. Hooijer, D. A. in *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y., Howell, C. F., Isaac, G. L. & Leakey, R. E. F.) 209-213 (University of Chicago Press, 1976).
5. Eisenmann, V. in *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y., Howell, C. F., Isaac, G. L. & Leakey, R. E. F.) 225-233 (University of Chicago Press, 1976).
6. Hooijer, D. A. *Zool. Verh., Leiden* **142**, 1-75 (1975).
7. Hooijer, D. A. *Zool. Verh., Leiden* **148**, 1-39 (1976).
8. Eisenmann, V. *Bull. Mus. natn. Hist., nat., Paris* **438**, 69-87 (1977).
9. Eisenmann, V. *Bull. Soc. geol. Fr.* **21**, 277-281 (1979).

LINDSAY ET AL. REPLY—We regret that the important palaeontological contributions of Dr Eisenmann and others were slighted in our references. This was unintentional, but resulted from a bias towards selection of references with a chronological rather than a palaeontological message.

Certainly, the paper by Eisenmann and Brunet¹ on the co-occurrence of *Equus* and *Hipparion* at Roccaneyra is an important palaeontological contribution for recognition of the appearance of *Equus* in Europe. Our study was initiated with the expectation that the record of *Equus* at Montopoli would be demonstrably earlier than that at Roccaneyra, and we were more impressed with the proximity of their age assignment than with the palaeontological identity of the equids at Roccaneyra and Montopoli.

We cited Maglio² as an early review of East African biochronology in which faunal levels were characterized, including the *Mesochorus limnetus* zone, with the appearance of *Equus*. Correlation of this faunal sequence had been questioned because of similar faunas with conflicting radiometric limits in the Shungura and Koobi Fora Formations—that conflict was resolved after further work on the radiometric dating, as discussed by Drake³. Our emphasis was on resolution of the conflict, and we concluded that the appearance of *Equus* in deposits of the Omo Basin, east of Lake Turkana, was contemporaneous with that at Olduvai Gorge. Unfortunately, we did not acknowledge the palaeontological contributions of Hooijer⁴, Eisenmann⁵, Churcher⁶, and others.

We think there might be a strong tendency for reviewers to cite other reviews, and similarly for analytical contributions to cite other analytical contributions. In spite of this, we recognize and appreciate the numerous palaeontological, radiometric, and stratigraphic studies of many researchers whose work we drew on for our review.

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1. Eisenmann, V. & Brunet, J. in *Int. Colloquium on the Problem 'The Boundary Between Neogene and Quaternary'* **4**, 104-122 (Moscow, 1973).
2. Maglio, V. J. *Nature* **239**, 379-385 (1972).
3. Drake, R. E., Curtis, G. H., Cerling, T. E., Cerling, B. W. & Hampel, J. *Nature* **283**, 368-372 (1980).
4. Hooijer, D. A. in *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y., Howell, C. F., Isaac, G. L. & Leakey, R. E. F.) 209-213 (University of Chicago Press, 1976).
5. Eisenmann, V. in *Earliest Man and Environments in the Lake Rudolf Basin* (eds Coppens, Y., Howell, C. F., Isaac, G. L. & Leakey, R. E. F.) 225-233 (University of Chicago Press, 1976).
6. Churcher, C. S. *Zool. Meded., Leiden* **55**, 265-280 (1980); *Can. J. Earth Sci.* **18**, 330-341 (1981).

Pulsar birthrates

NARAYAN AND VIVEKANAND¹ have obtained a minimum estimate for the birth rate of pulsars in the Galaxy of 1 pulsar per $(100^{+100}_{-30})f$ yr, where $f(=K^{-1}) \leq 1$ is the beaming factor. They have obtained this estimate from the flow rate in period space, without recourse to the spin-down age $\tau = P/2\dot{P}$. At the same time, their estimated number of pulsars in the Galaxy is $N = 1.4 \times 10^{5 \pm 0.3}/f$, and they find that τ is a good measure of age for $\tau \leq 0.5 \times 10^6$ yr. Their method is elegant, but I find it hard to trust their result quantitatively, for the following reason.

Their birth rate N implies a mean pulsar age $N/\dot{N} \approx 4 \times 10^7$ yr which is some 10 times larger than the average age determined² both from the fraction of young pulsars ($\tau < 10^6$ yr, for which τ is held to be a good measure of age) and from the kinematic ages $z/\dot{z}$, and also³ from the histogram of spin-down ages. It would imply that τ underestimated the true age. However, according to our understanding of pulsars, τ measures their age for a dipole-coupling to their surroundings, and can only lose its property of an age indicator in the presence of some overtaking ageing mechanism (such as spin alignment⁴), in which case it would overestimate the true age.

If the birth rate derived by Narayan and Vivekanand can be trusted vaguely, it means that τ is not always as large an overestimate of age as suggested by kinematic ages. Such a trend does not surprise me in view of the two populations of pulsars which are expected if pulsars are born in binary systems⁵. A large fraction of all τ -old pulsars may be 'elder twins' born with a large τ_0 ($> 10^6$ yr, instead of $\leq 10^3$ yr), and for which τ is not a significant overestimate of age. At the same time, if pulsars are in general the younger twins, the birth rate of neutron stars should be approximately twice that of pulsars.

Another word of caution concerns the beaming factor whose value is often assumed to be 0.2. This estimate follows from the assumption of an almost circular beam cross-section, and independently from the fact that most supernova remnants lack a central pulsar. However, supernova remnants housing a pulsar would almost certainly have a filled-centre appearance, that is, be plerions, whereas shell-type remnants are expected⁶ to contain binary system neutron stars (like W50 around SS433). Moreover, pulsar beams may well have banana-shaped cross-sections, with $f \approx 1$. A beaming factor f near unity is likewise indicated by the high occurrence rate of interpulses ($\approx 5\%$) if the latter come from the opposite magnetic pole.

With these modifications and ref. 2 in

mind, I arrive at a galactic neutron star birth rate of 1 in 20 ± 10 yr.

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1. Narayan, R. & Vivekanand, M. *Nature* **290**, 571 (1981).
2. Lyne, A. G. *IAU Symp.* **95**, (1980).
3. Kundt, W. *Naturwissenschaften* **68**, 63 (1981).
4. Kundt, W. *Astr. Astrophys.* **98**, 207 (1981).
5. Kundt, W. *Naturwissenschaften* **64**, 493 (1977).

NARAYAN AND VIVEKANAND REPLY—It is known¹ that the radio luminosities of pulsars decrease with age. Therefore, the observed sample of pulsars is biased towards the more luminous younger pulsars. Consequently, the average age of pulsars determined² from the observed fraction of young pulsars ($\tau < 10^6$ yr) underestimates the true mean pulsar age. The bias is probably even more stronger in the kinematic ages determined from z/z because proper motions have been reliably obtained only for relatively luminous pulsars. On the other hand, in our analysis, we have accounted for radio luminosity selection effects and thereby obtained an approximation to the

complete galactic population of pulsars. Consequently, our result $N/\dot{N} \approx 11 \times 10^6$ yr is unbiased and a more reliable estimate of the mean pulsar age than the earlier approaches which typically gave 4×10^6 yr. Note, however, that age is not a very relevant parameter in our analysis, which is based entirely on pulsar flow in the $P-\dot{P}$ diagram (pulsar period to its time derivative). Moreover, the concept of mean pulsar age itself may not be very meaningful or useful if pulsars belong to more than one class with widely different active lifetimes.

Regarding the beaming factor K , we consider that the 5% occurrence of inter-pulses is consistent with a circular beam cross section and a value of $K = 5$ rather than with banana-shaped cross sections and $K = 1.0$.

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1. A. G. Lyne, R. T. Ritchings & F. G. Smith *Mon. Not. R. astr. Soc.* **171**, 579–597 (1975).
2. A. G. Lyne. *IAU Symp.* **95**, (1980).

Solar-flare produced ^3He in lunar samples

RAO AND VENKATESAN¹ have reported a method for deducing the absolute solar cosmic ray (SCR) proton fluxes in the past few million years using SCR proton-induced ^3He in the top surface layers of Moon rocks. To quote the authors: "even if there are minor diffusion losses, this method holds good as long as the losses are not depth dependent". I would like to point out that Moon rocks studied in this laboratory which are similar to those used by Rao and Venkatesan show severe ^3He diffusion losses, up to 99% (refs 2–6), and the losses show strong depth dependence^{3–6}. Another problem arises from the directly implanted solar flare ^3He found in the top few millimetres of these Moon rocks^{3–6}. This ^3He component shows depth dependence and is about one order of magnitude higher in concentration than the SCR proton-induced ^3He , therefore masking it in the top few millimetres of rock³. The rocks which we have studied, 68815 and 65315, were excavated from south ray crater, have GCR and SCR exposure ages of 2 Myr, contain plagioclase as the major mineral and therefore are similar in many characteristics to the rocks studied by Rao and Venkatesan, 61016 and 64435. The near constancy of $^3\text{He}/^{21}\text{Ne}$ ratios which they suggest as an indication that depth dependence diffusion effects are not significant can also be misleading as the ^{21}Ne diffusion pattern shows in our rocks a similar depth dependence to the ^3He and

therefore the $^3\text{He}/^{21}\text{Ne}$ ratio might stay quite constant. We believe that Rao and Venkatesan were unable to observe the two effects, depth dependence diffusion and the directly solar flare-implanted ^3He because first, only three layers of different depths were taken from each rock, and second, the layers were too thick (≥ 1.5 mm). We can judge from our fine sampling, nine for each rock in the range 0–15 mm and ~ 0.2 mm thick layers in the first 0–1 mm of range, that neither the diffusion pattern nor the SCR-implanted ^3He would have been observed using the sampling technique of Rao and Venkatesan. I therefore consider ^3He to be the wrong isotope for the use proposed unless the effects mentioned are taken into account.

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1. Rao, M. N. & Venkatesan, T. R. *Nature* **286**, 788–790 (1980).
2. Yaniv, A. & Marti, K. *Meteoritics* **15**, 390 (1980).
3. Yaniv, A. & Marti, K. *Astr. Phys. J. Lett.* **247**, L1–L4 (1981).
4. Yaniv, A., Kirsten, T. & Richter, H. *Astr. Phys. J. Lett.* (submitted).
5. Yaniv, A. & Kirsten, T. *Proc. 12th lunar planet. Sci. Conf.*, 1224–1226 (1981).

RAO AND VENKATESAN REPLY—We point out that we discussed these issues in detail elsewhere¹. Relevant points are briefly outlined below.

Our inferences regarding ^3He losses are based on experimental results in rocks

61016 and 64435. The general agreement (within a factor of two) of exposure ages based on SCR-produced ^{21}Ne and ^{38}Ar and ^3He , with the surface exposure ages based on particle tracks, could not have been observed if almost total loss of ^3He had taken place by diffusion in these samples. Further, the diffusion effects of ^3He and ^{21}Ne are mass-dependent. Yaniv may not be observing such mass-dependent effects because 99% of the gas has been lost from his samples. The depth-dependent diffusion will lead to variations in the $^3\text{He}/^{21}\text{Ne}$ and $^3\text{He}/^{38}\text{Ar}$ ratios with depth, which is not borne out by the near-uniformity of these observed ratios in our samples. (Details are discussed in refs 1, 2.)

The peak temperatures to which some of these rocks were exposed on the lunar surface is ~ 370 K and the average temperature is 210 K; He loss can be partial but not almost total, at such temperatures, in these samples.

In some lunar plagioclases, ^3He was lost and in some it was well retained. In a sensitive Gas ion-probe analysis of ^4He in plagioclases, Müller *et al.* observed large amounts of ^4He in two plagioclases (P4 and P2), with a capability of retention similar to that of ilmenite, whereas in some other plagioclases from soil 76501, no ^4He was found. Shock effects also seem to be involved.

The occurrence and composition of directly implanted solar flare He and Ne in lunar samples is important. We are investigating the composition of solar flare Ne in these samples by selective chemical etching and subsequent stepwise thermal release methods. The long-term solar flare Ne composition seems to be similar to fractionated solar wind⁴. The long-term solar flare He composition is not well known, but the contemporary solar flare $^3\text{He}/^4\text{He}$ ratios (measured from spacecraft) vary from 1 to 0.01 and differs between flares⁵. Most of the ^3He -rich flares are very weak and their contribution to the total He seems to be limited. It is not clear whether the long-term solar flare He composition is similar to the fractionated solar wind as in the case of Ne or varies drastically as observed in contemporary flare studies. Further controlled experiments are under way to understand these complexities.

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1. Venkatesan, T. R. *et al. Proc. 11th lunar planet. Sci. Conf.* 1271–1284 (1980).
2. Rao, M. N. & Venkatesan, T. R. *Nature* **286**, 788–790 (1980).
3. Müller, H. W. *et al. Proc. 7th lunar Sci. Conf.* 937–951 (1976).
4. Nautiyal, C. M. *et al. Proc. 12th lunar planet. Sci. Conf.* (1981) (in the press).
5. Ramaty R. *et al. NASA. Tech. Rep.* 79660 (1978).

BOOK REVIEWS

Chinese puzzles

John Maddox

THE time has gone when people could bring conversation in the West to a halt by saying that they were just back from China. Indeed, the conversational gambit is dangerous, for there is as likely as not to be somebody in the gathering who can boast of having found a better hotel, or of having been allowed to visit Chinese Mongolia or Tibet. Yet China remains a puzzle, not only because mutual travelling is still only a trickle but because of language and the sheer strangeness of China as seen from the West and, no doubt, vice versa.

Science in Contemporary China is, in the circumstances, a welcome book even though its defects, sometimes serious and often irritating, also command attention. The book is a compilation of travellers' tales from the late 1970s brought back from China by 26 scholars of various disciplines, many of whom seem to have made two or three journeys under the auspices of the Committee on Scholarly Communication with the People's Republic of China, itself an offshoot of the National Academy of Sciences in Washington but funded, for this purpose, by the National Science Foundation. But since most of the contributions are well laced with handlists of laboratories, and the names of their directors, the book is likely to be most used as a kind of Baedeker — a guide by which intending travellers from the West can plan their journeys. That is not something to be scorned.

The most glaring flaw is that the book, according to its editors and Professor Walter Rosenblith, the chairman of the supervisory committee, is uneven. In many ways, that is an understatement. Some contributions are merely handlists. Others sensitively relate the condition of some discipline now (or in 1978 or 1979) with the great events of the Cultural Revolution — which then seems more than ever a calculated madness. A more forgivable defect of the book is that most of the journeys on which it is based were made in that dreadful period when the Cultural Revolution had come to an end but when Mao Zedong (according to the *pin-yin* transliteration) was still nominally in the saddle. Some travellers were plainly mystified by what was happening around them, at least until the changing scene was crystallized and made familiar by the great National Science Conference in Peking (sorry, Beijing) in March 1968.

Travellers to foreign lands must be wary of the three most obvious traps — those of generalizing from impressions, of writing patronizingly and of writing with stars in their eyes. This bunch of travellers has been

Science in Contemporary China. Edited by Leo A. Orleans with the assistance of Caroline Davidson. Pp. 599. ISBN 0-8047-1078-3. (Stanford University Press: 1981.) \$35.

reasonably circumspect. Now and again, first impressions enliven the separate catalogues of information — Leo Goldberg was obviously shocked to learn of the ups and downs of the Purple Mountain Observatory (Nanjing) from which the astronomers were driven after the Japanese occupation in 1937. The general first impression that Chinese researchers use less sophisticated equipment than their colleagues in the West has, however, been carefully confirmed; and there is a thorough account of what is being done by the Chinese Academy of Science to put things right.

For the most part, devotional prose has also been avoided. Fang Yi, the vice-premier with responsibility for science and technology in 1968, made a great impression on several of the travellers as did Deng Xiaoping (deputy premier at the same time) on the smaller number who had the luck to meet him. Some seem to have regarded the national plan announced in March 1978 as having the force of Mosaic tablets, but collectively the travellers have given a good account of how the plan is having to be matched against economic and social reality.

Now and again, the travellers have fallen into the trap of patronizing those whom they observed. The impression is heightened by the way in which China is so often called the PRC. One writer asks how it can be that the Chinese choose to lavish surgical skill on intricate operations when they have so few Western-style doctors per 10,000 population. Even, in an extended footnote, Joseph Needham (the author of the monumental *Science and Civilization in China*) is patronized because his book is not "philologically sound". But these are minor points.

For the rest, this Baedeker, based though it is on 26 snapshots of China, is full of absorbing information. Not merely can one learn that C.N. Yang (the field theoretician) is the son of K.C. Yang, who took a PhD in mathematics in Chicago in the 1920s, but that Chen Jingrun was the first to prove (in 1966) that, above a certain size, every even number can be written as the sum of a prime number and another which is either itself prime or the product of two prime numbers; that China is heavily committed to superconducting technology for reasons not readily understood; that

the commitment to high-energy physics derives from Mao's interest in the philosophical implications (but that the 50 GeV accelerator will not now be finished until 1985); that there is a project to synthesize a transfer-RNA molecule and it appears to be customary, when an active compound has been isolated from some traditional medicinal herb, to synthesize the pure chemical so as to avoid the associated side-effects. China's need of military electronic equipment is urgent. Computers still languish.

Collectively, the snapshot is clear. But what is happening to the people? The Cultural Revolution lasted for 15 years, almost a generation, and university posts were either not filled or filled with the wrong people. *Science in Contemporary China* records in several places that Chinese hosts were aware of the problems foolishly created, and that they were doing their best to put things right. How quickly, one must wonder, will they succeed?

Anecdotally, this book provides some evidence of change in the right direction. I was pleased to find in it the name (almost unrecognizable behind the *pin-yin*) of a graduate student at Manchester many years ago. He was so homesick that he travelled every other week to Liverpool for a decent meal in St George's Square. In 1953, he caught Mao's student boat back to China. We were surprised to learn that as an F-centre expert he planned to help with the "building of a dam"; only afterwards did we understand that it was a project to do with weapons. But when seen at the height of the Cultural Revolution, he was working as a kind of filing clerk at the Academy. Now, it seems, he has been rehabilitated, the head of a division of an institute in Beijing.

Only occasionally do the contributors have the time to reflect on what they describe, but Saunders Mac Lane (who writes about mathematics) does best. He gives an account of how his delegation chose to argue with their Chinese hosts about the virtues of mathematics — is it elegance ("beauty") or utility that matters most? The encounter was apparently rowdy, and the question unresolved. The reason seems to have been a difference of idiom — which is not merely a name for a pretty turn of words. What remains to be learned, from the West, about China is whether present puzzles will melt away with the passage of time or whether, on the other hand, the idiomatic gulf will prove to be unbridgeable. □

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Physics for philosophers and philosophy for physicists

D. ter Haar

Superposition and Interaction: Coherence in Physics. By Richard Schlegel. Pp.302. ISBN 0-226-73841-8. (Chicago University Press: 1981.) \$22.50, £13.50.

THIS is an interesting book but — to my mind — very much a curate's egg. The first question which comes to mind is: for whom is the book meant? The author himself states that although at times he had thought of philosophers as his audience, his primary prospective audience is among physicists or other physics-minded scientists "who have some technical knowledge of special relativity theory and of quantum theory — as given, say, in introductory courses in those topics in American universities". The next question is: what is the message which the author wants to convey? The answer is that he wishes to suggest that there is a fundamental coherence in physics which can be shown especially to exist when we consider the two main pillars of what is still called "modern" physics, namely, the special theory of relativity (now 76 years old) and non-relativistic quantum theory (in its late sixties). The suggestion is that in both of these theories superposition (that is, the fact that if one has two separate solutions of the equations, a line or combination of them is also a solution) plays an essential part and interactions with an observer are a deciding factor. This is orthodox as far as quantum theory is concerned, but takes some swallowing when we come to special relativity. However, while this book does not give any compelling reasons — in my view, not even aesthetic reasons — to accept this thesis, there do not seem to be any experimental data which contradict it. This is, perhaps, not surprising, as no *experimenta crucis* are suggested to decide for or against the author's views.

Let me first of all list those aspects of the book which I found most admirable and from which I benefited most. For a careful and well-informed reader it contains much that is illuminating. In particular, I found the first and last chapters of great interest. The discussion in the first chapter of the contents of classical physics — going back to Aristotle — and of the importance of models in theoretical physics in general and in classical physics in particular is excellent. The last chapter containing a "summer-time conversation" between two physicists, the wife of one of them and a philosopher gives a cogent and on the whole clear account of most of the important points made in the main body of the book.

The second volume in the series *Genetic Engineering* (for a review of Vol.1 see *Nature* 292, 480) has recently appeared. *Genetic Engineering 2* contains four contributions on gene evolution, genomic libraries, restriction enzymes and gene cloning in yeast. Prices are: £9.80, \$24.

One might object to quantum mechanics as a *deus ex machina* and the relegation of God to a superior watchmaker — miracles seemed to have no place in the Universe inhabited by these four people — but these are minor objections. Another excellent discussion is the one of the clock paradox, where the author follows a suggestion made to him by Einstein himself and uses three uniformly moving inertial systems to resolve this paradox.

I now come to a number of points where I feel the author has seriously diminished the usefulness of this mainly philosophical treatise, especially for younger physicists. I would hesitate to give the book to my own pupils without a number of caveats, since in a number of places the theory given is wrong (or at least so poorly formulated as to be seriously misleading). In the account of measurement in quantum theory there is, in my opinion, insufficient emphasis on the difference between the preparation for a measurement and the measurement itself. However, the most fundamental error — and one which crops up several times — is in the discussion of pure states and mixtures. A mixed state *cannot* be represented by a wavefunction, but needs a density matrix. The concept of a density matrix — so central in the theory of measurement — is, in fact, nowhere mentioned in this book.

Other points where the text is, to say the least, misleading are where the author states that the Maxwell equations are linear (homogeneous) differential equations for which the superposition principle holds, without stating that this is only true for the Maxwell equations *in vacuo*. It is also confusing to stress de Broglie's introduction of the relations between energy/momentum

and frequency/wavevector as against Schrödinger's introduction of the wave equation in order to find a close relation between special relativity and non-relativistic quantum theory. After all, we still do not have a complete relativistic wave equation, but only approximate equations such as the Dirac and Klein-Gordon equations which are valid in the not-very-relativistic domain.

In a book which is mainly philosophical in tone, one would expect careful use of language and it is disappointing to find a number of sloppy expressions. It is probably too much to expect that a careful distinction is made between utilize ("to make useful, turn to account" according to the *OED*) and use, but a vital point is missed when it is stated that "the Greek word *atomos* means 'individual'." As an important issue in the book is the distinction between macroscopic and microscopic systems, somewhere a careful definition of those terms should be given and the dividing line drawn between them — or it should be stated that it is often impossible to draw such a line. The general problem of what are physically meaningful questions and which are mainly philosophical ones, although hinted at several times, could also have been considered more carefully. For instance, I feel strongly that in a book of this kind there should be a clear discussion of the fact that a wavefunction only has a meaning in as far as it can predict the outcome of an experiment — this, after all, makes quantum theory a theory which can be falsified. □

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What price the diversity of species?

A.D. Bradshaw

Conservation and Evolution. By O.H. Frankel and Michael E. Soulé. Pp.327. ISBN hbk 0-521-23275-9; ISBN pbk 0-521-29889-X. (Cambridge University Press: 1981.) Hbk £25, \$49.50; pbk £7.95, \$17.95.

THE natural ecosystems of the world are disappearing at a frightening speed. Tropical rain forests are being destroyed at a rate of 4.7 ha min⁻¹ and will be gone within 50 years. Every year sees a further 6 million hectares of arable land. Although some areas will be almost impossible to cultivate, the ingenuity of people pressed for space must not be underestimated; and even if cultivation is not possible, hunting, logging and grazing will inevitably increase drastically beyond their present high levels.

Against this scenario, what will be left of

the wealth of plants and animals on this planet? Some species, *r* selected, of small size and with tolerance of man-made environments, will certainly survive. There are plenty of species left in England and China. But the great diversity will inevitably be reduced. This book is a critical analysis of what is likely to happen, written by two well-known population geneticists, highly experienced between them in both wild and cultivated species.

If species are to be conserved at all, the authors argue that they must be conserved without loss of genetic variability. For outbreeding organisms, from considerations of the effects of inbreeding/mutation/selection equilibria the authors conclude that a minimum effective population size of 50 is necessary.

But if conservation is to include the possibility of continuing evolution then the minimum population size is 500. Once this conclusion is reached the problems of conservation become apparent. The minimum territory of a pair of mountain lions is 52 km²; to conserve this species requires an area about twice the size of Yellowstone National Park. There are therefore few reserves in the world that are large enough to ensure the survival of the dominant carnivores, and there is no doubt the majority are doomed to disappear.

Yet nature reserves present the only possible solution. In which case there are criteria of population genetics and biology which must be observed if reserves are to be designed to function properly. Size is crucial according to island biogeographical theory, but so is migration. As a result there are arguments that small reserves are possible if there are connecting corridors. But the authors point out that this will only work in a limited number of cases.

Even the *r* selected species, notably our crop plants and their wild relatives, have problems. It is crucial that we maintain genetic diversity to satisfy our present and future needs for food production. This means genes to cope with specific individual problems such as day length or disease, and genes by which we can build up co-adapted complexes for characters such as yield. The authors show that these genes are scattered in wild and cultivated material, often in places that cannot be predicted.

For conservation and continued evolution, a diversity of populations must be maintained, if possible in natural conditions. So far, because of geographical expansion from migration and trade, the diversity in these species has actually increased. But now the success of modern agriculture and plant breeding has sent the process into reverse, and whole groups of cultivars, such as the Welsh upland wheats and barleys, have completely disappeared. The solutions endorsed by the authors are various: *in situ* and *ex situ* dynamic conservation, and static conservation by storage, of which seed storage is perhaps the most efficient. But they do not offer any easy solutions to the problem of what to store, except to emphasize the need for international co-operation.

This book is a remarkable survey of a problem which is crucial to us all. Not only does it list over 600 references but it achieves a remarkable synthesis and perspective, based on a masterly understanding of theory as well as practice. It is enormously readable because of its simple and enthusiastic style. It is critical reading for plant and animal breeders, and all ecologists, so that they can better argue with politicians about what has to be done, before it is too late. □

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Liquid crystal measurement

J. W. Emsley

Physical Properties of Liquid Crystalline Materials. By W.H. De Jeu. Pp.133. ISBN 0-677-04040-7. (Gordon and Breach: 1980.) \$35.25.

EVERY schoolboy knows that there are liquid crystal display devices, but a relatively few graduates are familiar with this "fourth state of matter". There is certainly a need for introductory texts on the physical properties of these materials, to supplement George Gray's excellent book, *Molecular Structure and the Properties of Liquid Crystals* (Academic, 1962), which covers their general properties. This short book by De Jeu is the first of what promises to be a series, edited by Professor Gray, on liquid crystals. It has, however, a misleading title since it does not cover all physical properties and is confined to discussing only the simplest mesophase, the nematic. This does have the advantage of making it a book that will be read rather than one too complex and long to appeal to those interested in other aspects of liquid crystals.

Five physical properties are discussed: magnetic susceptibility, refractive index, dielectric permittivity, elastic constants and viscosity coefficients. Each topic is introduced by a discussion of the basic physics, which makes heavy demands on the background knowledge of the reader.

There follows a survey of methods of measurement and a discussion of the work which has been done. The author draws heavily on his own research and does not attempt a general review, but this again adds to the readability of the book and conveys a sense that much remains to be done.

Physicists, chemists and engineers interested in liquid crystallinity will welcome this book as providing a short, readable account of the five physical properties. It is written primarily with experimentalists in mind and it has a particularly useful chapter on sample preparation. It is less successful at explaining the significance of the results, mainly because of a deliberate decision not to get involved with the details of the various theoretical models developed in recent years. Restricting the scope of the book to those topics in which the author has been directly involved was a wise decision, for although one might wish for more discussion of cholesteric or smectic phases, or a more critical review of theory, what is included is discussed with an authority derived from a long experience in the laboratory. □

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Consolidating fungal biochemistry

P.G. Mantle

Lipid Biochemistry of Fungi and Other Organisms. By J. D. Weete with contributions by D.J. Weber. Pp.388. ISBN 0-306-40570-9. (Plenum: 1981.) \$45, £28.35.

THIS volume is a reincarnation of the authors' previous book, *Fungal Lipid Biochemistry*, published by Plenum in 1974. The text has been completely rewritten and revised but still centres on the distribution and biochemistry of fungal lipids — fatty acids, acylglycerols, sterols, phospholipids, aliphatic hydrocarbons and sphingolipids. New aspects covered in the present volume include a brief history of fungal lipid research, more comprehensive treatments of lipid taxonomy and substrate conversion into stored lipid, a description of the multifunctional yeast enzyme for fatty acid synthesis, the biosynthesis of polyphenols and carotenoids, and a review of the role of fungal lipids in sporulation and spore germination. The bibliography has been correspondingly increased and, where appropriate, reference is made to contrasting bacterial, plant and animal systems.

A few minor typographical errors can be

recognized and I was somewhat surprised to find myself credited (Table 3.7) with research in the field of phycomycete fatty acids. Nevertheless, the authors should be congratulated on condensing the essence of about 1,000 research papers into a lucid text supplemented liberally by comprehensive tables and figures.

It is often only when someone gives the time and care to review a subject that the gaps in our knowledge of an otherwise diffuse topic become well defined. Fungal lipid metabolism is a case in point. Thirty years ago this book would have been simply a pamphlet listing some of the oils contained in fungi. Biochemistry has since moved so fast that the lipid composition of representatives of the major groups of fungi is now fairly well documented and the biosynthetic routes are generally understood. It is, however, notable that although regulatory mechanisms for fatty acid synthesis have been well researched, the regulatory interactions involving lipids in whole-organism metabolism are largely unknown. This is perhaps not surprising since it is bound to be very complex. Nevertheless, it is such areas of knowledge which

will be invaluable in fully understanding fungi as organisms. This is particularly relevant to species which interact parasitically with other organisms or which have the ability to produce complex secondary metabolites, whether suitable for exploitation as pharmaceuticals or agrochemicals or whether hazardous on account of their propensity to act as mycotoxins. Often these functions are closely associated with lipid metabolism, frequently yielding metabolites from a common pool of key intermediates.

In an age when biochemistry is concentrating so much on the molecular level, however exciting, it would appear that metabolic biochemistry is being neglected. This book will be valuable in focusing attention on fungal biochemistry — students, teachers and researchers in the field of microbial biochemistry will do well to consult it. □

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Academic synergism

J.B. Butterworth

Universities in Partnership. By I.C.M. Maxwell. Pp.480. ISBN 0-7073-0270-6. (Scottish Academic Press: 1980.) £15.

Universities in Partnership describes the first 25 years of the work of the Inter-University Council for Higher Education Overseas, from the time when it was set up by the British universities in 1946 to assist in the creation of new universities in colonial territories which were rapidly becoming independent. Gradually the IUC was called upon to fulfil a wider role in helping these new institutions to develop their academic potential and train their staff. More recently, it became an important vehicle in facilitating better two-way communication between the universities in the UK and some 35 others in developing countries, an important partnership, based in equality, giving benefits to both sides by a number of different forms of relationship of which links between departments have become particularly important.

This record of the universities with which the IUC has had association is impressively comprehensive and portrays their development as seen from the standpoint of the IUC. One is grateful to the author, Mr Maxwell, for his immense work of distilling the records of the IUC and, from a voluminous amount of material, producing such a readable and full account. At a time when the IUC is becoming the IUPC and merging with the British Council, it is important that all of this material should be available within the covers of one volume.

The book is divided into three parts, the first of which is particularly interesting in

that it gives a short and attractive account of the basic theories upon which the work of the IUC has been based, and of the way in which those theories or principles have changed with time. In Part 2, "The Foundation and Growth of the Universities", Mr Maxwell presents the universities in considerable detail, much of which comes from the archives of the IUC. The book seems to change gear between Part 1 and Part 2, the earlier section dealing in a lively way with the principles and policies which evolved over the 25 years. The second part, no doubt because it is dealing with each individual university and is condensed from the IUC records, does not always say what one might wish to hear. In the development of any institution people are crucial, and the end of the period covered by Mr Maxwell was particularly important because indigenous Vice-Chancellors were taking over from expatriates; one would have liked to know more about those individuals whose influence had been crucial in the development of particular universities.

The book, of course, is intended to be a history of the IUC and not of the individual universities with which it was associated. But too close an adherence to the reports of commissions and the decisions of Senates and Councils may not always reveal the reality which is the university. Moreover, it would have been interesting if Part 2 or Part 3 on the role of the IUC had explored more fully some of the important themes referred to earlier in the book, especially the historical growth of the concept of a developmental university. The ideas and plans already current in the 1960s, as the Kericho Conference in 1966 bears evidence, were having and were to have considerable effect upon the new universities in developing countries. A prime example is the concept of an umbrella university such as Malawi, taking within itself virtually all post-school education. Another is the single university catering for a number of different countries, evolving from the University of the West Indies, to the University of Botswana, Lesotho and Swaziland, and the experience there acquired being applied in the University of the South Pacific. The concept of a developmental university advanced rapidly after 1970 and it might have been advantageous if Mr Maxwell had not stopped abruptly at that date, for a deeper exploration of these themes could have given guidance and illumination to university development both now and in the future.

Nonetheless, we should be grateful to the author for the valuable work of reference he has made available to us. Those who consult it about particular events in the history of any of the universities are unlikely to be disappointed; it is an extensive and objective record. □

J.B. Butterworth is Vice-Chancellor of the University of Warwick, was Chairman of the IUC from 1968 to 1977, and becomes Chairman of the IUPC from 1981.

Of bugs and brains

Howard C. Berg

Bacterial Chemotaxis As a Model Behavioral System. By Daniel E. Koshland. Pp. 193. ISBN 0-89004-468-6. (Raven: 1981.) \$19.50.

BACTERIA, the most primitive free-living organisms, continuously monitor the concentrations of chemicals in their environment, decide whether these concentrations are rising or falling, shift gears in their rotary engines and swim toward regions that they find more favourable. The modern armament of genetics, biochemistry and biophysics has been brought to bear on this remarkable behavioural system, and some of the processes involved are now understood in molecular detail. Professor Koshland surveys this work in terms intended for both the educated layman and the specialist, striving, as he puts it, to bridge the gap between general interest and scientific accuracy. He then moves with some temerity to a discussion of the function and malfunction of the human brain, arguing that the biochemistry of bacterial chemotaxis is relevant to the nervous system of human beings themselves.

This is a provocative book. It speaks with authority about the interactions of ligands with receptors, receptors with signalers, and signalers with enzymes that methylate and demethylate. It describes models for signalling and adaptation that, although incomplete, have led to a number of fruitful biochemical studies. It contains lively speculations on necessity and chance, memory and learning, and even love and hate.

But I have serious reservations. This is a personal account. The book does not adequately treat pioneering work on bacterial motility and chemotaxis done outside Berkeley. Nor does it make any attempt to place in proper historical perspective the idea that the nervous system of higher organisms evolved from reactions that can be found in the most primitive living things, or the hope that a knowledge of the mechanisms of bacterial chemotaxis might contribute to our understanding of neurobiology and psychology. These concepts are not new. Neurobiologists already know that they need to learn more about the biochemistry of the brain. A biochemist of Professor Koshland's experience might have been expected to offer more insights into problems that they might profitably pursue.

I prefer to think of bacteria as objects of fascination in their own right. Indeed they may prove to be more sophisticated than cells of the human brain. Bacteria have been around for billions of years, and individuals in each generation have had to survive on their own merits. □

Howard C. Berg is Professor of Biology at the California Institute of Technology, Pasadena.

BOOKS RECEIVED

Mathematics

- BARAGOZZI, C. Vito Volterra Symposium on Mathematical Models. Lecture Notes in Biomathematics, Vol. 39. Proceedings of a Conference held at the Centro Linceo Inter-disciplinare, Accademia Nazionale dei Lincei, Rome, December 1979. Pp.417. Flexi ISBN 3-540-10279-5. (Springer-Verlag: 1980.) DM 48.50, \$28.70.
- DEVLIN, K. J. Sets, Functions and Logic. Basic Concepts of University Mathematics. Pp.90. Hbk ISBN 0-412-22660-X; pbk ISBN 0-412-22670-7. (Chapman & Hall: 1981.) Hbk np; pbk £2.95.
- GORDON, V. O. and SEMENTSOV-OGIEVSKII, M. A. A Course in Descriptive Geometry. Pp.376. ISBN 0-8285-1870-X. (Imported Publications, Chicago: 1981.) \$10.
- GREENSPAN, D. Arithmetic Applied Mathematics. Pp.165. Hbk ISBN 0-08-025047-5; flexi ISBN 0-08-025046-7. (Pergamon: 1981.) Hbk £11, \$25; flexi £5, \$11.25.
- GREENSPAN, D. Computer-Oriented Mathematical Physics. Pp.170. Hbk ISBN 0-08-026471-9; flexi ISBN 0-08-026470-0. (Pergamon: 1981.) Hbk £10, \$25; flexi np.
- KOLMAN, B. and SHAPIRO, A. College Algebra and Trigonometry. Pp.446. ISBN 0-12-417840-5. (Academic: 1981.) Np.
- LAKSHMIKANTHAM, V. and LEELA, S. Nonlinear Differential Equations in Abstract Spaces. International Series in Nonlinear Mathematics. Theory, Methods and Applications, Vol. 2. Pp.258. ISBN 0-08-02538-6. (Pergamon: 1981.) \$36, £15.
- PRIESTLEY, M. B. Spectral Analysis and Time Series. Vol. 1, Univariate Series. Pp.653. ISBN 0-12-564901-0. (Academic: 1981.) £49.60, \$119.50.
- PRIESTLEY, M. B. Spectral Analysis and Time Series. Vol. 2, Multivariate Series. Pp.890. ISBN 0-12-564902-9. (Academic: 1981.) £20.60, \$49.50.
- VAJDA, S. Linear Programming. Algorithms and Applications. Pp.150. Pbk ISBN 0-412-16430-2. (Chapman & Hall: 1981.) Pbk £4.50.

Astronomy

- HOPKINS, J. Glossary of Astronomy and Astrophysics. 2nd Edn. Pp.196. ISBN 0-226-35171-8. (University of Chicago Press: 1980.) \$17.50.
- PARISH, L. The Theory of Cosmic Aberration. A New Interpretation of the Hubble Red-Shifts. Pp.44. Pbk ISBN 0-904378-11-X. (Cortney Publications, Luton, England: 1981.) Pbk £3.50.
- RAINE, D. J. The Isotropic Universe: An Introduction to Cosmology. Monographs on Astronomical Subjects, 7. Pp.253. ISBN 0-85274-370-X. (Adam Hilger, Bristol/Heyden & Son, Philadelphia: 1981.) £19.50.
- SWIHART, T. L. Radiation Transfer and Stellar Atmospheres. Astronomy and Astrophysics Series, Vol. 12. Pp.130. ISBN 0-912918-18-7. (Pachart Publishing House, Tucson, Arizona: 1981.) \$24.

Physics

- HERMAN, M. A. (ed.). Semiconductor Optoelectronics. Pp.648. ISBN 0-471-27589-1. (Wiley: 1981.) £19.50, \$53.65.
- KAHNG, D. (ed.). Silicon Integrated Circuits Part B. Applied Solid State Science. Advances in Materials and Device Research. Pp.297. ISBN 0-12-002957-X. (Academic: 1981.) \$39.
- KRAUSE, F. and RÄDLER, K.-H. Mean-Field Magnetohydrodynamics and Dynamo Theory. Pp.271. ISBN 0-08-025041-6. (Pergamon: 1981.) £15, \$36.
- LATHAM, R. V. High Voltage Vacuum Insulation: The Physical Basis. Pp.245. ISBN 0-12-437180-9. (Academic: 1981.) £15.80, \$38.
- NICOLIS, G., DEWEL, G. and TURNER, J. W. (eds). Order and Fluctuations in Equilibrium and Nonequilibrium Statistical Mechanics. XVIIth International Solvay Conference on Physics. Nonequilibrium Problems in the Physical Sciences and Biology. Pp.374. ISBN 0-471-05927-7. (Wiley: 1981.) £32.75, \$68.75.
- POZHELA, J. Plasma and Current Instabilities in Semiconductors. Pp.301. ISBN 0-08-025048-3. (Pergamon: 1981.) £27, \$65.
- WEBB, G.-A. (ed.). Annual Reports on NMR Spectroscopy, Vol. 11A. Pp.282. ISBN 0-12-505311-8. (Academic: 1981.) £35, \$84.
- WILKINSON, D. (ed.). Progress in Particle and Nuclear Physics, Vol. 6. Nuclear Astrophysics. Proceedings of the International School of Nuclear Physics, Erice, March-April 1980. Pp.349. ISBN 0-08-127117-0. (Pergamon: 1981.) £35, \$81.

Computer Science

- BYWATER, R. E. H. Hardware/Software Design of Digital Systems. Pp.477. ISBN 0-13-383950-8. (Prentice-Hall: 1981.) £15.95.
- COOMBS, M. J. and ALTJ, J. L. (eds). Computing Skills and the User Interface. Computers and People Series. Pp.499. ISBN 0-12-186520-7. (Academic: 1981.) £19, \$46.
- MARS, P. and POPPELBAUM, W. J. Stochastic and Deterministic Averaging Processors. IEE Digital Electronics and Computing Series, 1. Pp.157. ISBN 0-906048-44-3. (Peter Peregrinus, Stevenage, Herts: 1981.) £18 (US) \$52.50 (elsewhere) £21.50.
- MONDS, F. and McLAUGHLIN, R. An Introduction to Mini and Micro Computers. Pp.132. Flexi ISBN 0-906048-6. (Peter Peregrinus, Stevenage, Herts: 1981.) £9 (US) \$27.50 (elsewhere) £11.
- ONOE, M., PRESTON, K. Jr. and ROSENFELD, A. (eds). Real-Time Parallel Computing. Image Analysis. Based on the Proceedings of the Japan-United States Seminar, held in Tokyo, Japan, October 1978. Pp.397. ISBN 0-306-40639-X. (Plenum: 1981.) \$45.
- PAKER, Y. Minicomputers. A Reference Book for Engineers, Scientists and Managers. Pp.505. ISBN 0-85626-188-2. (Abacus, Tunbridge Wells, U.K.: 1981.) £32.50.

SARGENT, M. I. and SHOEMAKER, R. L. Interfacing Microcomputers to the Real World. Pp.288. Pbk ISBN 0-201-06879-6. (Addison-Wesley Advanced Book Program: 1981.) Pbk \$14.50.

Earth Sciences

- ANIKOUCHINE, W. A. and STERNBERG, R. W. The World Ocean. An Introduction to Oceanography. 2nd Edn. Pp. 513. ISBN 0-13-967778-X. (Prentice-Hall: 1981.) \$19.95.
- ARMSTRONG, J.E.. Geological Survey Bulletin 322. Post-Vashon Wisconsin Glaciation, Fraser Lowland, British Columbia. Pp.34. Pbk ISBN 0-66-10709-0. (Geological Survey of Canada, Ottawa: 1981.) Pbk \$4.80.
- BELOUSSOV, V. V. Geotectonics. Pp.330. ISBN 3-540-09173-4. (Springer-Verlag: 1980.) DM 48, \$28.40.
- BORGESE, E. M. and GINSBURG, N. (eds). Ocean Yearbook 2. Pp.713. ISBN 0-226-06603-7. (University of Chicago Press: 1981.) \$35.
- DILABIO, R.N.W. Geological Survey Bulletin 323: Glacial Dispersal of Rocks and Minerals at the South End of Lac Mistassini, Quebec, with special reference to the Icon Dispersal Train. Pp.46. Pbk ISBN 0-660-10817-8. (Geological Survey of Canada, Ottawa: 1981.) Pbk \$4.80.
- FOLLETT, R. H., MURPHY, L. S. and DONAHUE, R. L. Fertilizers and Soil Amendments. Pp.557. ISBN 0-13-314336-8. (Prentice-Hall: 1981.) £16.20.
- HÅKANSON, L.A. Manual of Lake Morphometry. Pp.78. Flexi ISBN 3-540-10480-1 (Springer-Verlag: 1981.) DM 19, \$11.30.
- KASAHARA, K. Earthquake Mechanics. Pp.248. ISBN 0-521-22736-4. (Cambridge University Press: 1981.) £25.
- SNOWDON, L. R. Geological Survey Bulletin 291. Organic Geochemistry of the Upper Cretaceous-Tertiary Delta Complexes of the Beaufort-Mackenzie Sedimentary Basins, Northwest Territories. Pp.46. Pbk ISBN 0-660-10167-X. (Geological Survey of Canada, Ottawa: 1981.) Pbk \$4.80.

Biological Sciences

- AGARWAL, M. K. (ed.). Streptozotocin: Fundamentals and Therapy. Pp.312. ISBN 0-444-80302-5. (Elsevier/North-Holland Biomedical: 1981.) \$95, Dfl. 195.
- AHNE, W. (ed.). Fish Diseases. 3rd COPRAQ-Session. Pp.252. ISBN 3-540-10406-2. (Springer-Verlag: 1980.) DM 89, \$52.60.
- AINSWORTH, G. C. Introduction to the History of Plant Pathology. Pp.315. ISBN 0-521-23032-2. (Cambridge University Press: 1981.) £27.50.
- AUGUSTINAK, J. (ed.). Biological Implications of Protein-Nucleic Acid Interactions. Pp.667. ISBN 0-444-80292-4. (Elsevier/North-Holland Biomedical: 1980.) \$69.75, Dfl. 143.
- BALIAN, R. et al. (eds). Membranes and Intercellular Communication. Proceedings of Les Houches Summer School, Session XXXIII, July-August 1979. Pp.657. ISBN 0-444-85469-X. (North-Holland: 1980.) \$122, Dfl. 212.50.
- BENNETT, M. D., BOBROW, M. and HEWITT, G. (eds). Chromosomes Today, Vol. 7. Proceedings of the 7th International Chromosome Conference held in Oxford, England, August 1980. Pp.310. ISBN 0-04-575021-1. (Allen & Unwin, London: 1981.) £16.
- BERNSTEIN, I. A. and SEIJI, M. (eds). Biochemistry of Normal and Abnormal Epidermal Differentiation. Current Problems in Dermatology, Vol. 10. Proceedings of the U.S.-Japan Seminar, Boyne Mountain Lodge, Boyne Falls, Michigan, July-August 1979. Pp.442. Flexi ISBN 3-8055-1915-X. (Karger, Basel: 1980.) Flexi SwFr. 136, DM 163, \$81.50.
- BERTI, F. and VELO, G. P. (eds). The Prostaglandin System. Endoperoxides, Prostacyclin, and Thromboxanes. Proceedings of a NATO Advanced Study Institute, held September 1979 in Erice, Italy. Pp.428. ISBN 0-306-40645-4. (Plenum: 1981.) \$49.50.
- BEWLEY, J. D. (ed.). Nitrogen and Carbon Metabolism. Developments in Plant and Soil Sciences, Vol. 3. Proceedings of a Symposium held in Calgary, Canada, July 1980. Pp.248. ISBN 90-247-2472-4. (Martinus Nijhoff, The Hague: 1981.) Dfl. 75, \$39.50.
- BIRGE, E. A. Bacterial and Bacteriophage Genetics. An Introduction. Pp.359. ISBN 0-387-90504-9. (Springer-Verlag: 1981.) \$24.80.
- BITTAR, E. E. (ed.). Membrane and Structure Function, Vol. 4. Pp.246. ISBN 0-471-08774-2. (Wiley: 1981.) £23.85, \$50.
- BLASECKI, J. W. (ed.). Mechanisms of Immunity to Virus-Induced Tumors. Immunology Series, Vol. 12. Pp.368. ISBN 0-8247-1162-9. (Marcel Dekker: 1981.) SwFr. 134.
- BOCOCK, K. L. Radionuclides in Terrestrial Ecosystems. A Review of their Distribution and Movement. Pp.27. Flexi ISBN 0-904282-49-X. (Institute of Terrestrial Ecology, 68 Hills Road, Cambridge: 1981.) £2.
- BOURNE, G. H., DANIELLI, J. F. and JEON, K. W. (eds). International Review of Cytology, Vol. 70. Pp.351. ISBN 0-12-364470-4. (Academic: 1981.) \$42.
- BRANDENBURG, G. and WOLLMER, A. (eds). Insulin: Chemistry, Structure and Function of Insulin and Related Hormones. Proceedings of the 2nd International Insulin Symposium, Aachen, Germany, September 1979. Pp.752. ISBN 3-11-008-156-3. (Walter de Gruyter, Berlin and New York: 1980.) DM 70.
- BRITISH MUSEUM (NATURAL HISTORY)/CAMBRIDGE UNIVERSITY PRESS. Origin of Species. Pp.120. Hbk ISBN 0-521-23878-1; pbk ISBN 0-521-28276-4. Np.
- BRUCE, W. R. et al. (eds). Banbury Report 7. Gastrointestinal Cancer: Endogenous Factors. Pp.410. ISBN 0-87969-206-5. (Cold Spring Harbor Laboratory, Cold Spring Harbor: 1981.) \$65 (US) \$78 (elsewhere).
- BRYCE, C. F. A. Biochemical Education. Pp.219. ISBN 0-7099-0600-5. (Croom Helm, London: 1981.) £12.95.

BURGER, H. and DE KRETZER, D. (eds). *The Testis*. Pp.454. ISBN 0-089004-247-0. (Raven: 1981.) \$54.40.

FISCHER, A. and SMITH, E. F. *The Fischer-Smith Controversy: Are there Bacterial Diseases of Plants?* *Phytopathological Classics*, No. 13. Pp. 65. Pbk ISBN 0-89054-014-4. (The American Phytopathological Society, St. Paul, Minnesota: 1981.) Pbk \$8.50.

FISHER, R. *My Jungle Babies*. Pp. 181. Pbk ISBN 0-09-926000-X. (Arrow Books, London: 1981.) Pbk £1.50.

FITTER, A. H. and HAY, R. K. M. *Environmental Physiology of Plants*. *Experimental Botany*, Vol. 15. Pp. 340. Hbk ISBN 0-12-257760-4; pbk ISBN 0-12-257762-0. (Academic: 1981.) Hbk £19.60 (UK only) \$47.50; pbk £7.95 (UK only) \$19.50.

FOLLETT, B. K. and FOLLETT, D. E. (eds). *Biological Clocks in Seasonal Reproductive Cycles*. *Colston Papers*, Vol. XXXII. *Proceedings of the 32nd Symposium of the Colston Research Society held in the University of Bristol, March-April 1980*. Pp. 292. ISBN 0-85608-032-2. (Sciencetechnica, Bristol: 1981.) Np.

FRAME, G. and FRAME, L. *Swift and Enduring: Cheetahs and Wild Dogs of the Serengeti*. Pp. 242. ISBN 0-525-93060-4. (Elsevier-Dutton, New York: 1981.) \$16.50.

FRANKLIN, T. J. and SNOW, G. A. *Biochemistry of Antimicrobial Action*. 3rd Edn. Pp. 217. Hbk ISBN 0-412-22440-2; pbk ISBN 0-412-22450-X. (Chapman & Hall: 1981.) Hbk £14.50; pbk £7.50.

FREDRICK, J. F. (ed.). *Origins and Evolution of Eukaryotic Intracellular Organelles*. *Annals of the New York Academy of Sciences*, Vol. 361. Pp. 512. Hbk ISBN 0-89766-111-7; pbk ISBN 0-89766-112-5. (New York Academy of Sciences, New York: 1981.) Hbk and pbk \$99.

HENSEL, H. *Thermoreception and Temperature Regulation*. *Monographs of the Physiological Society*, No. 38. Pp. 324. ISBN 0-12-341260-9. (Academic: 1981.) £20.20, \$48.50.

HEPPER, F. N. *Bible Plants at Kew*. Pp. 64. Flexi ISBN 0-11-241171-1. (HMSO: 1981.) £2.95.

HERSCOWITZ, H. B. *et al.* (eds). *Manual of Macrophage Methodology*. *Collection, Characterization, and Function*. *Immunology Series*, Vol. 13. Pp. 560. ISBN 0-8247-1222-6. (Marcel Dekker: 1981.) SwFr. 175.

HORNBEIN, T. F. (ed.). *Regulation of Breathing, Part 1*. (In two parts). *Lung Biology in Health and Disease*, Vol. 17. Pp. 696. ISBN 0-8247-1014-4. (Marcel Dekker: 1981.) SwFr. 185.

HORZINEK, M. C. *Non-arthropod-borne Tagaviruses*. Pp. 200. ISBN 0-12-356550-2. (Academic: 1981.) £16.40, \$39.50.

HOWARD, A. N. and BAIRD, I. M. (eds). *Recent Advances in Clinical Nutrition*. 1. *Proceedings of the First International Symposium on Clinical Nutrition*, July 1980, Royal College of Physicians, London. Pp. 298. ISBN 0-86196-009-2. (John Libbey, London: 1981.) £18.

ISMAL, A. A. *Biochemical Investigations in Endocrinology*. *Methods and Interpretations*. Pp. 275. ISBN 0-12-374850-X. (Academic: 1981.) £14.20, \$34.50.

JÄGER, W., ROST, H. and TAUTU, P. (eds). *Biological Growth and Spread*. *Mathematical Theories and Applications*. *Lecture Notes in Biomathematics*, Vol. 38. *Proceedings of a Conference held at Heidelberg, July 1979*. Pp. 511. Flexi ISBN 3-540-10257-4. (Springer-Verlag: 1980.) Np.

Psychology

DICKSON, W. P. (ed.). *Children's Oral Communication Skills*. Pp.394. ISBN 0-12-215450-9. (Academic: 1981.) \$29.50.

FREEDMAN, J. L., SEARS, J. L. and CARLSMITH, J. M. *Social Psychology*. 4th Edn. Pp.686. ISBN 0-13-817783-X. (Prentice-Hall: 1981.) £12.95.

HOFER, M. A. *The Roots of Human Behavior*. *An Introduction to the Psychobiology of Early Development*. Pp.331. Hbk ISBN 0-7167-1277-6; pbk ISBN 0-7167-1278-4. (W. H. Freeman: 1981.) Hbk \$22.50, pbk \$11.50.

HOFFMEISTER, F. and STILLE, G. (eds). *Psychotropic Agents*. Part I: *Antipsychotics and Antidepressants*. *Handbook of Experimental Pharmacology*, Vol. 55/1. Pp.734. ISBN 3-540-09858-5. (Springer-Verlag: 1980.) DM232, \$136.90.

HOUSTON, J. P. *et al.* *Essentials of Psychology*. Pp.529. Flexi ISBN 0-12-356858-7. (Academic: 1981.) \$14.95.

LA BARBA, R. C. *Foundations of Developmental Psychology*. Pp.545. ISBN 0-12-432350-2. (Academic: 1981.) \$18.95.

LOCKER, D. *Symptoms and Illness*. *The Cognitive Organization of Disorder*. Pp.193. ISBN 0-422-77460-X. (Tavistock, London: 1981.) £12.

MENDELS, J. and AMSTERDAM, J. D. (eds). *The Psychobiology of Affective Disorders*. *Pfizer Symposium on Depression*, Boca Raton, Florida, February 1980. Pp.220. Flexi ISBN 3-8055-1400-X. (Karger, Basel: 1980.) SwFr. 39, DM47, \$23.50.

WALKER, C. E. *et al.* *Clinical Procedures for Behaviour Therapy*. Pp.400. ISBN 0-13-137794-9. (Prentice-Hall: 1981.) £12.95.

WINGFIELD, A. and BYRNES, D. L. *The Psychology of Human Memory*. Pp.429. Flexi ISBN 0-12-759650-X. (Academic: 1981.) Flexi \$10.95.

History of Science

BAXBY, D. *Jenner's Smallpox Vaccine: The Riddle of Vaccinia Virus and its Origin*. Pp.214. ISBN 0-435-5407-2. (Heinemann Educational, London: 1981.) £8.50.

HANLE, P. A. and CHAMBERLAIN, VON DEL. (Eds). *Space Science Comes of Age*. *Perspective in the History of the Space Sciences*. Pp.194. Hbk ISBN 0-87474-508-X; pbk ISBN 0-87474-507-1. (National Air and Space Museum Smithsonian Institution, Washington: 1981.) Np.

MOLELLA, A. P. *et al.* (eds). *A Scientist in American Life: Essays and Lectures of Joseph Henry*. Pp.136. Pbk ISBN 0-87474-641-8. (Smithsonian Institution, Washington, D.C.: 1981.) Pbk \$6.95.

NEWELL, H. E. *Beyond the Atmosphere*. *Early Years of Space Science*. *The NASA History Series*. Pp.197. (Superintendent of Documents, U.S. Government Printing Office, Washington: 1980.) Pbk np.

STEARN, W. T. *The Natural History Museum at South Kensington. A History of the British Museum (Natural History) 1753-1980*. Pp.414. ISBN 434-73600-7. (William Heinemann, London: 1981.) £15.

Anthropology

ANDERSON, D. C. and SEMKEN, H. A. Jr. (eds). *The Cherokee Excavations*. *Holocene Ecology and Human Adaptations in Northwestern Iowa*. Pp.277. ISBN 0-12-058260-0. (Academic: 1980.) \$23.

HEALY, P. F. *Archaeology of the Rivas Region, Nicaragua*. Pp.382. ISBN 0-88920-094-7. (Wilfred Laurier University Press: 1981.) \$13.50.

MUCKELROY, K. *Discovering a Historic Wreck*. *Handbooks in Maritime Archaeology*, No.1. Pp.47. Pbk ISBN 0-905555-51-1. (Trustees of the National Maritime Museum, London: 1981.) Pbk £1.20.

ROBINSON, W. S. *First Aid for Marine Finds*. *Handbooks in Maritime Archaeology*, No.2. Pp.40. Pbk ISBN 0-905555-52-X. (Trustees of the National Maritime Museum, London: 1981.) Pbk £1.20.

STYLES, B. W. *Faunal Exploitation and Resource Selection*. *Early Late Woodland Subsistence in the Lower Illinois Valley*. *Northwestern University Archaeological Program: Scientific Papers*, No.1. Pp.312. (Northwestern University Archaeological Program, Evanston, Illinois: 1981.) Pbk \$12.

General

GILLIESPIE, J. H. and TIMONEY, J. F. *Hagan and Bruner's Infectious Diseases of Domestic Animals*. *With Reference to Etiology, Pathogenicity, Immunity, Epidemiology, Diagnosis, and Biologic Therapy*. 7th Edn. Pp.851. ISBN 0-8014-1333-8. (Cornell University Press: 1981.) \$39.50.

GLOVER, D. M. *Genetic Engineering Cloning DNA*. Pp.78. Flexi ISBN 0-412-16170-2. (Chapman & Hall: 1980.) £2.45.

GOLDSPINK, D. F. (ed.). *Development and Specialization of Skeletal Muscle*. Pp. 155. Hbk ISBN 0-521-23317-8; pbk ISBN 0-521-29907-1. (Cambridge University Press: 1981.) Hbk £18, pbk £8.95.

GOODERS, J. *The Bird Seeker's Guide*. Pp.208. Pbk ISBN 0-233-xxxx-8; pbk ISBN 0-233-97380-X (André Deutsch: 1981.) Hbk £6.95; pbk np.

HARRIS, E. and HARRIS, J. *The Guinness Book of Trees*. *Britain's Natural Heritage*. Pp.160. ISBN 0-85112-303-1. (Guinness Superlatives, Enfield, Middlesex: 1981.) £4.50.

MIDDLETON, W. E. K. *Radar Development in Canada: The Radio Branch of the National Research Council of Canada 1939-1946*. Pp.148. ISBN 0-88920-106-4. (Wilfred Laurier University Press: 1981.) \$9.75.

MUCKELROY, K. *Discovering a Historic Wreck*. *A Handbook Offering Some Advice On What To Do When You Find An Archaeological Site Under Water*. *Handbooks in Maritime Archaeology*, No. 1. Pp.47. Flexi ISBN 0-905555-51-1. (National Maritime Museum, Greenwich, London: 1981.) £1.20.

MURRAY, J. and MONRO, M. (eds). *Uranium and Nuclear Energy: 1980*. *Proceedings of the 5th International Symposium held by the Uranium Institute, London, September 1980*. Pp.359. ISBN 0-86103-041-9. (Westbury House, Guildford, England: 1981.) Np.

OPPENHEIM, D. *Small Solar Buildings in Cool Northern Climates*. Pp.139. Flexi ISBN 0-85139-596-1. (Architectural Press, London: 1981.) £12.95.

PERSINGER, M. A. *The Weather Matrix and Human Behavior*. Pp.327. ISBN 0-03-057731-4. (Praeger/Holt-Saunders, New York: 1980.) £18.

PIAGET, J. *Experiments in Contradiction*. Pp.310. ISBN 0-226-66779-0. (University of Chicago Press: 1981.) £13.20.

POWELL, J. *Aircraft Radio Systems*. Pp.255. ISBN 0-273-08444-5. (Pitman Publishing: 1981.) £18.75.

ROCHE, H. *Premiers Outils Taillés D'Afrique*. Pp.264. Pbk ISBN 2-90116114-6. (Société d'ethnographie: 1980.) Pbk np.

ROSE, M. *Curator of the Dead*. *Thomas Hodgkin (1798-1866)*. Pp.148. ISBN 0-7206-0527. (Peter Owen, London: 1981.) £9.50.

ROSENTHAL-SCHNEIDER, I. *Reality and Scientific Truth*. *Discussions with Einstein, von Laue, and Planck*. Pp.148. ISBN 8143-1650-6. (Wayne State University Press: 1980.) Np.

SAGER, N. *Natural Language Information Processing*. *A Computer Grammar of English and Its Applications*. Pp.399. ISBN 0-201-06769-2. (Addison-Wesley: 1980.) £37.50.

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SPALENY, J. (ed.). *Proceedings of the 3rd Internationale Conference Bioindicators Deteriorationis Regionis*, 12-16 September 1977, Lobice near Prague, Czechoslovakia. Pp.410. (Academia, Praha: 1980.) Pbk np.

TRAN THANH VAN, J. (ed.). *Elementary Constituents and Hadronic Structure*. Vol. 1. *Proceedings of the 15th Rencontre De Moriond, Les Arcs-Savoie, France*, 1980. Pp.702. ISBN 2-86332-006-8. (Editions Frontieres, Dreux: 1980.) Np.

TURNER, G. L. E. *Collecting Microscopes*. Pp.120. ISBN 0-289-70882-6. (Studio Vista, London: 1981.) £6.95.

WEIRZBICKA, A. *Lingua Mentalis*. *The Semantics of Natural Language*. Pp.367. ISBN 0-12-750050-2. (Academic: 1981.) \$54.

WOLF, J. A., CAHEN, M. and DE WILDE, M. (eds). *Harmonic Analysis and Representations of Semi-Simple Lie Groups*. *Mathematical Physics and Applied Mathematics*, Vol.5. *Lectures given at the NATO Advanced Study Institute on Representations of Lie Groups and Harmonic Analysis, held at Liège, Belgium, September 1977*. Pp.495. ISBN 90-277-1042-2. (Riedel: 1980.) Dfl. 125, \$66.